The influence of crop nutrition on the quality of onion bulbs destined for export markets

by

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Declaration of Originality

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Thesis Abstract

The key export onion markets demand consistent supply of high-quality bulbs with long storage life from Tasmania. Supplying these market requirements challenges our body of knowledge relating to bulb quality. This study sought to address the potential links between crop nutrition and bulb quality by surveying thirty-four commercial onion crops across seven soil types. Plant tissue concentrations were recorded through key growth and development stages from two true leaf to harvest. These results were then related to yield and bulb quality attributes through multivariate analyses. The findings were compared with existing literature on onion bulb production and in many cases, provides new information on elemental tissue concentrations at a number of growth stages not previously reported.

The survey established linkages between element concentration in plant tissue and bulb quality, particularly apropos to skin loss. Here a relationship of bulb moisture content below 87.6% together with tissue concentrations of molybdenum lower than 0.047 ppm was associated with a decrease in skin loss. In contrast, nitrate levels greater than 20 ppm were associated with higher levels of skin loss and this effect was exacerbated if bulb tissue sulphur concentrations also exceeded 0.34%.

Expanding on the quality linkages established from the initial survey, factorial experiments were then undertaken across four sites with two cultivars to explore the interaction of applied sulphur, molybdenum and nitrogen on onion plant elemental composition and bulb skin loss. Bulb robustness was assessed by subjecting harvested bulbs to multiple handling assessments over a five-month period. Amending the base fertiliser programme with ammonium sulphate increased sulphur

concentrations in the bulb tissue of both cultivars, and nitrogen levels in Regular Creamgold. Supplementation with foliar applied molybdenum also increased concentrations of this element within this cultivar.

This study has complemented existing knowledge and added new data for some onion growth stages not previously reported. This improves scientific understanding of the range of nutritional element concentrations found in high yielding onion crops and has provided evidence that consideration of plant nutrition not only applies to crop yield, but also to the quality of the onion bulbs produced.

Thesis introduction and scope

"If thinking is an intellectual response to a problem, the absence of a problem leads to the absence of thinking"
Levitt, 1975

"…… by this rationale, onion growers all over the world do lots of thinking!"
Steve McArthur, 2011

Introduction

Onion bulbs produced in Tasmania, Australia, are predominately exported to the Northern Hemisphere to meet supply gaps in counter-seasonal markets. To be competitive in these markets against other imported bulbs, the Tasmanian industry is based on a low-cost production system with bulbs stored at ambient air conditions before and during export.

An understanding of onion plant growth and development and its interaction with agronomic and environmental factors is fundamental to managing the production of high-quality bulbs (Agnieszka et al. 2017). Crop nutrition is an important component of a sustainable production system (Boyhan et al. 2014) including crop amendment timing and fertiliser composition (Lierop, Martel & Cescas 1980). Management decisions require the consideration of the onion growth stage (Fageria & Moreira 2011) soil type, including profile composition (Cotching et al. 2004), weather and overall production goals (Abdalla & Mann 1963) to ensure that both bulb yield and quality are maximised.

A substantial gap remains within scientific literature to identify the effect fertilisation has on onion skin quality and to enable the consistent production of robust onion bulbs for export destinations. This chapter will focus on nutritional requirements for export

bulb onions, the influence various elements have on onion physiology, and current capacity to interpret plant tissue tests for those elements during growth.

Taxonomy

Bulb onions (*Allium cepa* L.) belong to the monocot order Asparagales, family Alliaceae, and are predominately cultivated for their edible swollen leaf base (bulb). Species domesticated for food production within the genus *Allium* are collectively referred to as alliums and are unique amongst vegetable crops as they are only cultivated for their edible leaf bases (Bennett 1993). Other key edible alliums include leeks (*Allium ampeloprasum* L.), garlic (*Allium sativum* L.) and chives (*A. schoenoprasum* L.). Artefacts recovered from ancient Egyptian tombs show onions and garlic have been used as food as far back as 3200 B.C. and are noted as important food ingredients in the Bible and Koran (Schwartz 2008).

Of the edible alliums, the single bulb onion dominates commercial crop production, though bulb shape and colour vary widely as does leaf shape and size. The single bulb onion is commercially cultivated across the world from temperate to tropical climes. In the year 2014 onions were cultivated across 3.6 million hectares in over 175 different countries with an annual production of approximately 77 million metric tonnes (F.A.O.).

Onion growth and development

Onions are biennial plants comprised of leaves that arise alternately from the meristem with the older leaves on the outside and the younger leaves on the inside of the stem (Abdalla & Mann 1963). The key growth stages of onion plant development have been well described and illustrated by Rabinowitch (1998) and Brewster (2008).

Following planting and emergence of the primary root, the epigeal germination of the onion seedling leads to the cotyledon forming a “hook” shape above the soil. The cotyledon then senesces as the first true leaf appears. As leaf number continues to increase from the four to six true leaf stage, the first true leaf senesces followed by the second true leaf. Through the vegetative stage, onions will not have more than ten leaves at any one time (Brewster 2008) with total leaf count varying between ten and seventeen (Lancaster et al. 1996).

Prior to bulbing, each onion leaf is comprised of a photosynthetic leaf blade and a non-photosynthetic leaf sheath that may form either skin or scale (Abdalla & Mann 1963). Leaf blades that were initiated prior to bulbing continue to grow to full length (Lancaster et al. 1996). At the onset of bulbing, the formation of leaf blades is repressed` and bladeless sheaths are produced. The inner leaf and bladeless bulb sheaths then swell by cell enlargement to form scales, the storage tissue of the bulb. Following the formation of several bladeless sheaths associated with bulbing, several small bladed leaves arise from the neck of the mature bulb. Hence the onion bulb consists of three distinct types of leaves produced in sequence (Abdalla & Mann 1963). As onion scales continue to thicken, they form the characteristic bulb with defined ‘shoulders’, while desiccation of the outer most scales occurs to form skins (field skins).

Increasing temperature and photoperiod are the primary drivers of bulb initiation although the commencement of bulb development can also be influenced by other environmental stimuli and agronomic factors (Brewster 1989). Onion cultivars are clinal, and consequently, photoperiod and temperature dictate a cultivar’s optimal latitudinal range for production (Rabinowitch & Brewster 1990). Aligning crop growth and development with the appropriate daylength is achieved by adjusting the time of sowing to meet requirements of a specific cultivar (Figure 1). Using photoperiodic

requirement, cultivars can be grouped into three production regions and are classified as either short, intermediate or long day-length varieties. Within this, some subgroups are also recognised (Rabinowitch & Currah 2002). Short-day onions are grown at latitudes below 30 degrees. Intermediate-day varieties are grown between 30 and 45 degrees as autumn, winter and spring-sown crops. These varieties bulb in late spring to early summer and are ready for harvest during summer to mid-autumn. Long-day varieties are grown between 45 to 60 degrees latitude, are sown in spring and bulb in mid to late-summer. Varieties sown out of their designated latitude are unlikely to develop normal bulbs unless unusual microclimate conditions prevail (Boyhan & Kelley 2007).

Figure 1. Latitudes and daylength indication for onion cultivar selection (Mapworld 2018).

As bubs approach maturity, the pseudostem collapses (tops) and roots begin to senesce with both events providing an indication of when to lift the bulbs out of the ground. The timing of this process is a critical step that determines bulb skin quality (Wright & Grant 1997) and under Tasmanian conditions, research has shown crops should be lifted when eighty to ninety percent of onions tops have fallen over (Gracie

et al. 2006). Mechanical removal of the onions from the soil termed ‘lifting’ uses a tractor-drawn implement with a revolving bar that undercuts the root system and deposits windrowed bulbs on a machine rolled bed surface. Windrowing covers the bulbs with foliage, which protects the bulb from intense sunlight and subsequent sunburn during bulb curing. In-field bulb curing techniques employed depend on the production system and climate. These curing techniques include; permitting tops to fully desiccate and bulb skins to cure *in situ* in dry regions (Eshel et al. 2014); undercutting of roots but leaving bulbs *in situ* and then lifting bulbs and harvesting once the tops have desiccated (Vaughan 1960); or lifting bulbs when the canopy starts to collapse (but is still green) and arranging them in windrows on the ground to cure.

The technique of in-field curing is the traditional and most cost-effective approach to curing onion bulbs. This is made possible in Tasmania by a moderate climate during summer. Although in-field curing is the most cost-effective method, substantial summer rainfall can cause considerable skin staining and potentially render bulbs unsuitable for sale (Wright, Grant & Triggs 2001). During in-field curing the bulb diameter increases slightly while also “rounding out”, losing any deformation caused by close neighbouring onions (Brewster 2008).

In Tasmania, the duration required for skins to cure is affected by many factors such as solar radiation, air temperature, humidity, soil moisture, and diurnal relative humidity (RH) gradients (Clark & McDonald 1977; Rotz 1995). These environmental factors in conjunction with crop characteristics of biomass and bulb neck diameter influence the curing process (Brewster 2008). The duration of the curing process is further dependent on windrow depth and with favourable conditions including dry soil; relatively rapid skin curing is attainable. While less favourable conditions such as periodic rainfall on large crop windrows delays the curing process and can increase

bacterial disease and likelihood of Botrytis infection. Commonly in Tasmania a period of approximately 14 to 30 days of in-field curing is required for skins to mature and to allow bulb necks to dry before mechanical harvest commences.

By the time machine harvest commences the top has fully senesced and the onion neck dried and sealed (Wright, Grant & Triggs 2001). Mechanical removal of the dry onion tops occurs during harvest in Tasmania with the dry leaf material left in the field and the cured bulbs then transported to store. Although Australia and many other industrial countries harvest systems are mechanically based, large quantities of onions are still hand harvested in less industrialised regions (Rabinowitch & Currah 2002).

Other methods of curing are also utilised across the onion growing regions of the world. To overcome the unfavourable environmental factors during in-field curing, growing regions such as the UK cure bulbs in artificial conditions using forced air. This process involves storing freshly harvested onions in aerated bins and drying the bulbs in an enclosed area for 3 – 6 weeks at a temperature of approximately 28ºC with RH at 65–75%. Although more expensive than in-field curing, artificial drying can reduce the risk of skin staining and fungal growth possibly resulting in improved bulb appearance (Downes, Chope & Terry 2009). Control of RH during the storage process can affect several factors such as the number of skins that form. This is underpinned by the maintenance of the ideal RH range (Gubb & MacTavish 2002). A high RH percentage can accelerate pathogen attack of the bulb when the moisture content of the air rises above the moisture balance with the skin. High RH can contribute to root development of the bulb, skin loss from base plate extension (Gubb & MacTavish 2002) and premature breaking of dormancy from root development (Abdalla & Mann 1963).

Onion bulb curing is a formative process to achieve a complete dry outer skin. As well as aesthetically improving bulb colour which can be influenced by temperature conditions during curing, skin formation can also assist with disease reduction and prevent shrivelling of the bulb from moisture loss in the outer scales.

Tasmanian onion production

Tasmanian onion crops are typically established by directly planting seed into tilled soil. Onion crops can also be established using transplants or small bulbs <25mm called sets (Brewster 2008). Sets have a large reserve of stored assimilates compared to seed and like transplants can shorten growing days (Rabinowitch & Brewster 1990). Consequently, transplants and sets are preferred at higher latitudes (e.g. Japan and the UK) as they allow bulbs to finish over a short season (Rabinowitch & Brewster 1990).

As the Tasmanian export market has a long shipping time, onion growers have traditionally used open-pollinated (OP) cultivars with long dormancy. These cultivars share their genetics with the mainstay Southern Hemisphere seed lines, such as those sown extensively in New Zealand (Wright & Grant 1997) and South Africa (Comrie 1986). Since the commencement of the Tasmanian industry in the late 1960s the selection processes have yielded two key varieties; Early Creamgold (ECG) and Regular Creamgold (RCG). These are intermediate daylength, long keeping export varieties (Rabinowitch & Currah 2002) which are generally harvested in January to March (Autumn) and can be stored through until September (Spring) (Maier, Dahlenburg & Twigden 1990b). The ECG genotype has a sowing window of May/June and exhibits good tolerance to the premature production of a flowering stem (bolting). Bulb size generally ranges between 40 to 70mm in diameter. Attempting to produce

an ECG bulb with a diameter of more than 75mm under Tasmanian conditions can increase bulb defects, due to the potential for large loose necks and double centred bulbs. These traits are regarded as quality defects as they are unattractive to consumers. The RCG cultivar was developed for later sowing through July to September and exhibits a wider size tolerance producing up to a 100mm diameter bulb. Bulb size and timing of maturity are the primary logistical considerations for meeting market requirements (Lancaster et al. 1996).

Tasmanian onion crops are commonly sold prior to bulb maturation, and specific tonnages may even be contracted with customers prior to sowing. To ensure forward supply contracts can be met, growing crops are regularly assessed for quality, a common practice across onion bulb growing regions (de Visser & van den Berg 1998; Marino et al. 2013). In this context, the reputation of the producer and packer to consistently ship marketable onions is dependent on many variables that influence the supply chain, including environment, weather, and agronomic and nutrient management (Marino et al. 2013). These bulbs must have long dormancy and are required to withstand the rigours of handling, shipping and repacking upon arrival in the key export destinations of Europe, Japan or Asia (Allwright 1993; Gracie et al. 2012).

Onion bulb quality

The interaction between skin material, structural properties and moisture content play a significant role in determining skin quality (Gracie et al. 2006; Hole, Drew & Gray 2002). An important structural property contributing to skin strength is the thickness of the compressed scale (Hole, Drew & Gray 2002). This compressed scale is comprised of multiple layers of desiccated cellular tissue interspersed with vascular traces. Skin

thickness is determined by the quantity of cross-sectional structural material and moisture content of the scale tissue (Hole, Drew & Gray 2002). Onion skins are reported to range in thickness from 0.02 to 0.17mm (Gracie et al. 2012; Hole, Drew & Gray 2002). Thicker skins derived from higher cross-sectional cell numbers are desirable as they have a higher resistance to mechanical failure (Gracie et al. 2012). Failure of the skin tissue is also influenced by environmental factors, and both the breaking strength and modulus of elasticity can vary with different atmospheric conditions as scale moisture content changes with its surroundings and influences mechanical properties (Brewster 2008; Kumar, S, Imtiyaz & Kumar 2007). Until recently, the physiological basis for skin formation was not understood, however Galsurker et al. (2016) has provided evidence that the outer scale desiccates from the inside out, and that programmed cell death in the outer scales is fundamental to this process.

There is a common belief built over forty years of production in Tasmania that poor bulb quality traits, including skin loss, are partially attributable to crop nutrition protocols. This is not unusual as quality defects in other crops have benefited from the remedial application of macro and micronutrients. For instance;

- tip burn in lettuce and brown centre in potatoes have been reported as calcium deficiency symptoms (Collier & Tibbitts 1982; Poovaiah 1986);
- bitter pit development in apple trees with lighter fruit yield is reported as due to low calcium and high potassium tissue levels regardless of fruit size (Ferguson & Watkins 1989, 1992);
- Nitrogen application was linked to a decline in brown end in processing potatoes via a decrease in reducing sugars (Kumar, D, Singh & Kumar 2004).

Despite the likelihood of nutrition affecting bulb quality, establishing the response between crop amendment and changes in bulb attributes is challenging, particularly in field-based studies. Some of these relationships are known in short-day onion production, where supplemental calcium chloride was used to improve onion bulb firmness at harvest on low calcium soils (Coolong & Randle 2008). For long-day onion production, in at least one experiment increasing the level of phosphorus from zero to an optimum level increased flower bolts in overwintered onions, though at high levels this suppressed bolting in spring-sown onions (Greenwood et al. 2009).

Onion bulb pungency

Onion bulb quality is also judged by pungency (Crowther et al. 2005) and onions have been cultivated since antiquity primarily for this culinary property (Coolong & Randle 2003). The nature and origin of flavour compounds, particularly in onion and garlic, have been studied since the 1940s (Jones et al. 2004). These early studies demonstrated the distinctive pungency of onions is related to sulphur containing precursor compounds, collectively known as S-alk(en)yl cysteine sulfoxides (ACSO), (Bolandnazar, Mollavali & Tabatabaei 2012). The compounds responsible for pungency are formed by hydrolysis of the ACSOs by the enzyme alliinase (Randle, Kopsell & Kopsell 2002). Whilst tissue is intact, the reactive agents responsible for the production of these flavour compounds are separated between the cytoplasm and vacuole. Maceration of onion tissue exposes the ACSOs to alliinase (McCallum et al. 2005) which hydrolyses this precursor to form the thiosulfinates responsible for pungency, and the lachrymatory factor (LF) responsible for the tear-inducing action of cut onions (McCallum et al. 2007). The thiosulfinate's are unstable and over time are converted into other compounds that also contribute to other aspects of onion flavour (Randle, Kopsell & Kopsell 2002). Onion pungency and flavour attributes can vary in

response to growing media sulphur and nitrogen levels, which has implications for nutrient applications and flavour quality of the bulb (Coolong & Randle 2003).

Bulb storage

The bulb forming allium crops, which develop bulbs at longer daylength and higher temperatures are naturally suitable for storage (Petropoulos, Ntatsi & Ferreira 2016). Storage systems are different dependant on the location onions are grown. For instance, in contrast to Australian and New Zealand production systems, European and American onion growers commonly utilise on-farm air drying plenums and controlled atmosphere stores to meet market requirements (Brewster 2008; Tanaka, Matsuo & Egashira 1996)(pers comm. J Foskett, Suffolk U.K.). This system demands considerable knowledge of crop dormancy, management of disease and a prescribed supply chain upon exiting the store that matches the bulbs shelf life characteristics (Brewster 2008). Stored crops are sometimes tested in a “hot box” system to help predict bacterial load and manage storage requirements of bulb onions (VCS 2014).

Tasmanian production is counter-seasonal to Europe and onion export bulbs are stored in packing facilities at ambient air temperature for one to three months. The crop is then graded and packed in containers, either loose as bulk, as one-tonne bulk bags or conventional nets depending on customer requirement. To service this export market, shipping container doors are strapped ajar with custom fitted fans intermittently circulating fresh ambient air. These fans continue to circulate air during shipping from the Tasmanian dispatch port, to Melbourne through Singapore, the Suez Canal to Europe, or direct to Japan. Crops are discharged from the container and stored in ambient air facilities, with bulk shipped onions repacked before sale through supermarkets.

The shiny clean skin of a well-presented onion bulb will be the first thing a customer sees when considering purchasing Tasmanian onions in an overseas supermarket. Overall appearance is essential, especially as the imported product may be selling at a price point greater than locally produced out of storage crops (Brewster 2008; Hole, Drew & Gray 2002) (pers comm. Trofi Gmbh – Hamburg). The retention of bulb skins is essential but also difficult, as these can split and flake off if the onions are handled roughly at either harvest, removal from the store, grading, unloading at arrival or during repacking (Gracie et al. 2012). Bulbs that do not have an entire intact skin are deemed commercially unacceptable (Wright & Grant 1997) with quality standards for onions established independently (U.N.E.C.E. 2010)

In addition to skin loss, the breaking of dormancy also renders the bulb unsalable (Allwright 1993; Gracie et al. 2006). Various cultural practices are used to delay this process in onion bulbs although how these practices work is not fully understood. As examples, the timing and types of fertilisers applied to crops have been linked to shoot growth in storage (Rabinowitch & Brewster 1990). Komochi (1990), Vaughan (1960), Kumar (2007) and Bednarz (1986) showed crop inputs influenced the bulb shooting either directly or indirectly. Lower nitrogen applications were reported as a useful method for reducing the tendency of long daylength bulbs to sprout in storage (Sørensen & Grevsen 2001). Jones and Mann reported excessive nitrogen applied to onions through the growing season resulted in bulbs with thick, loose necks that did not store well (Rabinowitch & Brewster 1990). Wright (1993) similarly found higher than normal nitrogen applications can improve yields but increased the number of thick-necked bulbs of Creamgold onions grown in New Zealand.

Chemical intervention can also be used to increase the duration of bulb dormancy. Maleic hydrazide (MH) is an example of a chemical that has been widely used for this

purpose. MH is typically applied to crops prior to canopy collapse and has been shown to prolong storage life of dry bulbs (Ward & Tucker 1976). It has been reported that the sprout suppressant effect of correctly applied MH acts by lowering bulb respiration rate and can increase growth inhibitor activity (Isenberg et al. 1974). The efficacy of MH is dependent on crop stage, application timing and the appropriately applied dose resulting in a minimum concentration of 20ppm MH at the bulb centre (Brewster 2008) from ideal sprayer coverage of the target onion foliage (Petropoulos, Ntatsi & Ferreira 2016). Adverse effects from mistimed application can be evident with bulb scales exhibiting a soft spongy texture and prematurely sprouting product (Brewster 2008). Additional complications arise with aversion from export customers to accepting MH treated bulbs (Grevsen & Sorensen 2004).

The onion bulb is the plants natural storage vessel and is living, although appearing dormant, and moving towards sprouting and reproduction (Brewster 2008). True dormancy of onion bulbs is initially controlled internally regardless of the environment and is termed endo-dormancy (Chope et al. 2012). In transitioning from endo-dormancy suppression (or delayed) sprouting is determined by the storage conditions and is described as eco-dormancy. The mechanistic science controlling these physiological changes within the bulb are not fully understood (Chope et al. 2012) although the physiological and chemical changes of stored bulbs are reported to be affected by moisture loss, concentrations of flavour compounds and carbohydrates mobilised within the bulb for the energy requirement of the sprouting leaves (Agnieszka et al. 2017). These changes, reported to be linked to respiration and the concentrations of key metabolites (Chope et al. 2012) readies the bulb for the onset of sprouting. Onion cultivars vary considerably in their endo-dormancy and eco-dormancy, and thus storage potential (Grevsen & Sorensen 2004).

Onion nutrition

Our understanding of a plants requirement for specific nutrients has been developed over many centuries, and while progress has accelerated since earlier studies in the seventeenth century, there is still today controversy over the mechanisms of ion uptake, and the effectiveness of nutritional parameters in crop models (Le Bot, Adamowicz & Robin 1998). The process of nutrient acquisition by onions is complex with a constant interaction occurring between soil chemistry, temperature, fertiliser inputs, moisture and additional nutrients contained in irrigation water (Atwell 1999; Boyhan & Kelley 2007). This complexity is clearly epitomised in the title of Comrie's (1997) South African paper, '*Fertilizing of Onions, Art or Science*'.

The onion root system

The roots of alliums are comparatively thick and sparsely branched when compared with the root structure of most other crop species (Weaver & Bruner 1927). Despite onions having a longer growing season, they are noted as having the shallowest roots of commonly grown horticultural crops in contrast to carrot, lettuce and cabbage grown under similar conditions (Thorup-Kristensen 1999). In the United Kingdom, long-day onions are reported to have ninety percent of roots in the top 18 to 20cm of soil (Greenwood et al. 1982). The rooting depth of long and intermediate daylength onions has been reported at 30cm (Cotching 2009) with low root penetration (Thorup-Kristensen 1999). It is also likely that onions exhibit varying rooting depths attributed to water availability and soil structure (Rabinowitch & Currah 2002). The varied rooting depth between cultivars of different photoperiodic requirements has been known for some time, with Weaver and Bruner (1927) demonstrating different rooting depths between *Allium cepa* L. cultivars. Root architecture is also known to be different across *Allium* species, with the roots of Japanese bunching onion (*Allium fistulosum* L.)

developing fine lateral branches on the primary root axis which spread further and deeper into the soil than common onions (Rabinowitch & Currah 2002). Other alliums exhibit a root structure different again to common onions, notably pre-harvest leek (*Allium ampeloprasum* L.) for which root growth can exceed 60cm (Weaver & Bruner 1927). Common onions also lack root hairs when grown in soil or nutrient solution though do develop root hairs in a moist air environment (Brewster 2008).

To enable onion plants to prosper with their simple root structure, a symbiotic relationship has developed with vesicular-arbuscular mycorrhiza (VAM) (Rabinowitch & Brewster 1990). This relationship is suggested to have developed due to onions evolving from wild species in which VAM increased the root absorption surface (Brewster 2008). VAM fungi occur naturally in soils across the planet (Clarkson 1985; Furlan & Berniercardou 1989) and are present in almost all species, with exception of the notable omission of *Brassicaceae* and *Chenopodiaceae* species, which seem immune to colonisation (Rabinowitch & Brewster 1990). When roots of alliums are colonised by these fungi the hyphae penetrate between the cells of the root cortex, and external to the root provide a linkage to the soil solution for up to 8cm (Rabinowitch & Brewster 1990). This symbiotic relationship provides carbon metabolites from the plant to the VAM, while the increased absorptive surface area from the fungi increases nutrient uptake for the host allium.

To quantify the value of this relationship to the host onion, VAM can increase phosphorus availability similar to that of applying an additional 250 kg/ha^{-1} of superphosphate to a crop (Hayman & Mosse 1971). Juxtaposed with this, Rabinowitch and Brewster (1990) noted high concentrations of soil phosphorus can slow growth and reduce yield compared to crops not dependent on mycorrhiza. Hart and Forsythe (2012) also noted the VAM dependant *Allium porrum* L. showed both positive and

negative responses to inoculation depending on soil nutrient status (Hart & Forsythe 2012). In addition to phosphorus, the uptake of eleven macro and micronutrients may be influenced by VAM, and if so, manipulation of this relationship could lead to increased human health benefits due to better nutrient concentrations in food crops (Hart & Forsythe 2012).

Nutrient deficiencies in onion

With inflow rates of soil phosphorus required for young plants at a level three times higher than later growth stages (Brewster 2008) nutrient deficiency can be noted at an early stage of onion growth. At the seedling stage, in particular, this deficiency may be due to the small and shallow root system of the young onion plant (Rabinowitch & Brewster 1990). Onions with critically restricted nutrition at seedling development are not likely to recover and perform at the level of a well-supplied crop (Costigan, Greenwood & McBurney 1983). Further deficiency symptoms can become evident as onions grow through to the mid-growth stage. Bergmann (1992) describes onion foliage exhibiting visual symptoms to deficiencies of copper, potassium, nitrogen, phosphorous, sulphur and zinc. Schwartz and Mohan (2008) expand further describing symptoms of deficiency or toxicity of boron, magnesium, manganese and molybdenum. Onion toxicity symptoms have also been noted from excess chlorine concentrations in irrigation water (Bennett 1993) and have a low tolerance for saline soil conditions where concentrations greater than one dS/m EC may reduce gross yield proportional to concentration. Garlic (*Allium sativum* L.) is reported to have a higher tolerance to salinity than onions, possibly due to the faster root development of the clove as opposed to seed (Shannon & Grieve 1998).

Foliar fertiliser application

Supplementing onion nutrition by means of foliar fertilisers would seem the logical step to overcome a soil solute deficit. Applying nutrients for remedial correction of crop nutrition using foliar fertilisers has divided opinion amongst researchers. Bender (1993) states that trying to develop a highly soluble nutrient form that can be applied in sufficient amounts to change plant growth when applied to a waxy cuticle of a vertical leaf would be difficult. Any such application would do little more than runoff to the soil surface, ending up in the plant only through root absorption (Bennett 1993). Regardless of the mechanism, studies have demonstrated that copper sulphate, manganese sulphate, and zinc sulphate can be used successfully as a foliar spray on onions (Brewster 2008). Foliar sprays of zinc, boron and manganese also improved Egyptian onion yields over a two-season experiment whilst copper gave no yield improvement (Rabinowitch & Currah 2002). Whether these elements were absorbed through the leaf cuticle or acquired via the root system is debatable.

Nitrogen application

Meeting the nitrogen requirements of crops depends largely on factors such as climate, soil texture, cropping history, residual soil nitrogen and crop species (Buwalda & Freeman 1987). Few text books advising on nutrition are available for allium crop production and the industry's preeminent text notes the difficulty of efficiently supplying onions with N fertiliser (Brewster 2008). As an application guide, Brewster (2008) suggests an application range of N from 0 – 150 kg/ha^{-1}, leaving residual soil nitrogen after harvest. This recommendation was derived from modelling soil nitrate in the top 30 to 60 cm of the soil profile whilst considering seasonal variation due to differences in mineralisation and leaching. Nitrogen application on short day crops in the United

States can range from 157 to over 314 kg/ha^{-1} and it has been shown higher nitrogen levels may fail to increase yield, whilst lower nitrogen usage can accelerate bulb maturation (Coolong et al. 2005). Opposing this, South Australian research demonstrated ninety-five percent of maximum marketable bulb yield was achieved by applying nitrogen at 299 to 358 kg/ha^{-1}. Faster top senescence was observed for applications ≤100 kg/ha^{-1} and this low rate resulted in erect green tops that delayed harvest (Maier, Dahlenburg & Twigden 1990b). In Tasmania, top-dressed nitrogen was demonstrated to increase bulb yield but also influenced the response to potassium in onions (Laughlin 1989).

Some authors have posited that calculating the soil mineral N level before sowing does not contribute to the optimal nitrogen application rate necessary for producing high gross yields as mineralisation continues to occur during growth and development (Brewster 2008; de Visser, Van Den Berg & Niers 1995). Recovery rates of applied nitrogen have been reported by de Visser et al. (1995) to be quite low in long daylength onions at ca. 31% from 100 kg/ha^{-1}. This also aligns with a study by Greenwood et al. (1989) on a similar cultivar, and with a later investigation where higher application rates lowered the recovery rate (Greenwood et al. 1992). This was evidenced by an overall yield difference of only twelve percent between the highest nitrogen application of 200 kg/ha^{-1} and zero nitrogen. Here the amount of mineral nitrogen in the 0 to 30 cm soil zone explained more variation in the calculation of yield than the 30 to 60cm zone (de Visser, Van Den Berg & Niers 1995). Yield declines noted at high nitrogen rates in the experiment were possibly related to a reduction in plant density which declined from 103 to 95 plants m^2 (de Visser, Van Den Berg & Niers 1995). The authors suggested that an increase in fertiliser-induced salinity that affected seedling growth might also have been implicated in this yield loss. They also concluded that

soil mineralisation was the main source of nitrogen supply for the crop throughout the growing season (de Visser, Van Den Berg & Niers 1995).

Plant analysis in Allium crops

In a review of plant nutrition of horticultural crops, Le Bot et al. (1998) noted that the increased use of leaf analysis over the past sixty years has resulted in large collections of normal, deficient or toxic nutrient concentrations in plants. Each crop species has its own nutritional profile that may at first glance appear disconnected considering the similarity in metabolism between different species. However, it is evident from the modelling approaches that effective and sensible interpretation of analytical data for diagnostic purposes requires knowledge of a specific species critical nutrient concentrations over the whole growing cycle (Le Bot, Adamowicz & Robin 1998). Studies using plant analysis to establish concentrations of nitrogen, phosphorus, potassium, calcium, magnesium and sulphur in onion leaves have shown that an insufficient level of nutrition is as damaging to yield as excessive levels are to initiating metabolic disorders within the onion (Pankov 1984). Ekbladh (2007) also supports the use of plant analysis to evaluate the strategy of applying optimal nitrogen to crops whilst minimising environmental impact. Brewster et al. (1989) pointed out that regular monitoring of plant nutrient levels is pertinent to achieving this outcome. Despite this, use of plant analysis for this purpose has been limited due to lack of reliable reference values for the critical concentration needed for optimal growth (Ekbladh 2007).

Various methods are available to monitor the fluctuating plant nutrition levels of onion crops. The simplest is an in-field Cardy nitrate meter to measure NO_3-N concentration of undiluted xylem sap (Westerveld et al. 2004). Usually, a petiole or midrib section is used in horticultural crops due to the high concentration of nitrate in vascular tissue,

but onion roots can also be used as the quantity of nitrate in the root xylem is stable. Westerveld (2004) noted that establishing nitrate levels of onions using this method would require specific levels to be set for each crop and that a well-fertilised reference plot could help aid interpretation (Westerveld et al. 2004).

Laboratory sap analysis is an alternative monitoring method widely used in horticulture. Here, specified plant parts are collected from the field and delivered to the laboratory on the sampling day. A short time period between collection and analysis and a viable cool chain is crucial to enable minimal degradation of the material prior to analysis (Agvita 2013). The rapid turnaround provided by these laboratory services enables a grower or agronomist to change crop inputs in a timely manner if required. As sap flow dynamics, particularly xylem fluid movement is diurnal, it is usually recommended that collection of leaf material should be undertaken during the early morning (Agvita 2013).

This study based its analytical results on a third method, dry ash tissue analysis. With this method, defined plant parts are oven dried, ground, and then analysed using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). The advantage of this technique is that it is representative of the whole plants nutritional status. These analytical results are also directly equivalent to the guideline units published in *Plant Analysis an Interpretation Manual* (Reuter & Robinson 1997), this text being the primary and most comprehensive resource for onion agronomists.

Project scope

While there is a moderate body of research into onion nutrition, there are still no comprehensive references to enable the prediction and diagnosis of crop nutrient requirements specific to the key growth stages of onion crop development, and in particular, for Australian export onion crops. The location at which a study is conducted is of paramount importance as common onion cultivars vary their growth and development relative to photoperiod requirements, nutrition (Costigan, Greenwood & McBurney 1983) and field conditions (Petropoulos, Ntatsi & Ferreira 2016). For example, root depth is known to be reliant on cultivar selection and latitude of production (Weaver & Bruner 1927). Given the genetic diversity of alliums and the range of growing environments, it is necessary to determine if the current guideline values suit Australia's bulb production system.

The determination of nutritional needs for Tasmanian Creamgold onion varieties grown for storage and export are generally based on studies in the United States (Zink 1962, 1966). Though some data is referenced from Australian research conducted from the 1960's to 1980's (Reuter & Robinson 1997) the trial latitudes suggest the cultivars may have different daylength requirements and hence different nutrient recovery to intermediate-day onions grown in Tasmania. The validity of these tables for intermediate-day Southern Hemisphere production requires further research to understanding the optimal nutritional regime for Creamgold onion growth and development.

Production of Tasmanian onion bulbs for long-transit export adds layers of complexity to the production system in comparison to a short store domestic market, particularly as faults have more time to express. Understanding the effect crop nutrition has on

bulb skin defects and shoot emergence over an extended storage period is therefore clearly important for consistent quality and consumer acceptance. Remaining competitive in the counter-seasonal export market also demands a low-cost production system where it is only economically viable to store bulbs at ambient air conditions before and during export. As fresh market vegetable production is increasingly subject to customer quality assurance programs, it is also equally important to minimise the environmental impact of applied fertilisers (Agnieszka et al. 2017) while still maximising crop returns to growers and packers (Adamicki 2005).

The physiological status of onion bulbs at harvest determines their ability to withstand handling and storage effects (Petropoulos, Ntatsi & Ferreira 2016). Impacts on bulbs during both handling operations and storage conditions can lead to reduced shelf life and quality due to earlier sprouting (Hunt 2016). Cultural practices have also been shown to contribute to the prospective storage life of onion bulbs. As examples, the timing and types of fertilisers applied to crops have been linked to weight loss of bulbs and shoot growth in storage (Rabinowitch & Brewster 1990). Also, application of crop inputs has been shown to either directly or indirectly influence the storage life of onion bulbs Komochi (1990), Vaughan (1960), Kumar (2007) and Bednarz (1986).

Despite complex crop evaluation and post-harvest quality inspection, bulbs are selected and dispatched based on the tacit knowledge of packing staff, skin number and appearance. Notwithstanding the summation of experience and knowledge gained over years of local onion production, bulb quality still remains a major problem for Tasmanian exporters (Allwright 1993; Dennis et al. 2014). Gaining knowledge of plant tissue concentrations at key growth and development stages and combining these with crop assessment both pre and post-harvest, would assist with diagnosis of potential problems with crop quality for export markets.

Chapter 2 Summary; Plant nutrient levels during onion crop growth and development.

This chapter reports on an investigation to establish plant tissue concentrations from two true leaf to harvested bulb for Southern Hemisphere Creamgold onions, surveying 34 crops across northern Tasmania. No relationships between final bulb yield and plant tissue concentrations across all growth stages were detected. Although yield was unaffected, higher levels of skin loss were associated with higher levels of bulb sulphur, molybdenum, bulb moisture content and nitrate concentrations.

This chapter presents a comprehensive data set of elemental concentrations across six growth stages and supersedes previous information on onion nutrition in Tasmania.

Chapter 3 Summary; Does manipulating nutrient levels of onion plants affect skin and bulb quality?

Chapter three expands on the associations recorded between plant tissue concentrations of nitrate, sulphur and molybdenum and elevated levels of bulb skin loss. Experiments applying sulphur, molybdenum and ammonium sulphate were undertaken in four commercial crops of Early and Regular Creamgold cultivars. Amending the base fertiliser program with additional sulphur, molybdenum or ammonium sulphate led to higher plant tissue concentrations of the constituent elements, however, these elevated tissue concentration levels did not significantly affect the incidence of skin loss nor long-term bulb storage characteristics. Higher applications of sulphur resulted in higher pyruvate levels in mature bulb concentrations. Molybdenum application increased plant Mo concentrations alone, although when combined with sulphur application this response was reduced. Application of ammonium sulphate increased both plant nitrogen and nitrate concentrations across all growth stages while also decreasing soluble solids in mature bulbs. Experimental site had the greatest influence on bulb attributes and incidence of skin loss.

Plant nutrient levels during onion crop growth and development

Abstract

The reliable production of high-quality bulbs is fundamental to maintain market acceptance and ensure the longevity of the Tasmanian export industry, which to a degree is mediated by a crops nutritional program. As the actual nutritional requirements and their influence on bulb quality are only partially understood, this study evaluated the nutritional programme used for onion crop production in Tasmania, Australia, and reports on the range of elemental tissue concentrations during crop growth and development. Thirty-four commercial onion crops across seven soil types were surveyed to assess relationships between plant tissue nutrient concentrations, bulb yield and quality. Plant tissue concentrations for each element were defined against key growth stages and quantile trend lines are reported to provide a clearer assessment of crop nutritional demands and deficiencies.

This study established plant tissue concentrations from two true leaf to harvested bulb for Southern Hemisphere Creamgold onions. The plant tissue concentrations for the Group 3 nutrients were lower than published sufficiency thresholds. No relationship between final bulb yield and plant tissue concentrations across all growth stages were detected. Although yield was unaffected, higher levels of skin loss were associated with higher levels of bulb sulphur, molybdenum, bulb moisture content and nitrate concentrations. Data are presented on elements that have not had a comprehensive survey reported by growth stage previously in Australia. Linking onion quality to both pre and post-harvest conditions will enable further study into crop quality and possibly predictive suitability for export markets.

Introduction

Onion crop yield and quality outcomes require an understanding of the complex interactions among genotype (Rabinowitch & Currah 2002), crop stage of development (Boyhan, Torrance & Hill 2007), growing environment (Costigan, Greenwood & McBurney 1983) and agronomic practices (Bergmann 1992). In contrast to the complexity of this system, simple fertiliser regimes are routinely used to manage commercial crops, with these applications founded on a limited understanding of the dynamic plant-soil continuum (Rabinowitch & Currah 2002; Zink 1962, 1966). These regimes are loosely based on the total plant recovery at harvest, soil assessment, soil nutrient tests and on professional experience (Bennett 1993). Although soil nutrient tests are routinely conducted prior to sowing in commercial settings, these tests alone have not always been a good reflection of plant nutrient uptake and final crop yield and quality (Bennett 1993; Boyhan & Kelley 2007). Crops vary in relative nutrient requirements during growth and development (Reuter & Robinson 1997), highlighting the need to monitor and understand plant nutrient concentrations as they respond to a dynamic environment (Hochmuth et al. 2010).

Although ranges of onion tissue concentration have been identified for some normal plant functions, the link to crop quality outcome within these ranges is unclear and levels appear to be specific to location, species, genotype and stage of development (Costigan, Greenwood & McBurney 1983; Le Bot, Adamowicz & Robin 1998; Zink 1962, 1966). Due in part to the complexity of a plants nutrient acquisition and nutritional requirements, critical element values have not been comprehensively established for onions as they have for many other crops (Bennett 1993). As the onion grows, mobile elements may re-translocate from more mature and non-senescing leaves to young developing leaves. The mobility of each element influences the

location where deficiency symptoms are likely to be observed on the plant depending on the growing environment and crop stresses (Hartz & Hochmuth 1996). Regular monitoring of plant nutrient levels is pertinent to achieving a successful crop outcome (Brewster 1989). A lack of consistency in published recommendations for the quantity and timing of nutrient limits the confidence in where the optimal range lies (Ekbladh 2007).

Crop development and harvest timing can be influenced by nutrient application and delays or advances in maturity are indicated by varying fertiliser application rates and timing (Brewster 1989). In a U.S. study of short day type onions, nitrogen application rates varied widely and high nitrogen levels failed to increase yield whilst lower N usage accelerated bulb maturation (Coolong et al. 2005). Maier et al. (1990) indicated 95% of maximum marketable bulb yield was achieved in South Australian trials on siliceous sand by applying N at ca. 360 kg/ha^{-1}, and faster foliage senescence occurred from N at ca.100 kg/ha^{-1}. This lowest rate delayed canopy collapse and harvest when compared to the standard N treatment of ca. 360 kg/ha^{-1} (Maier, Dahlenburg & Twigden 1990b). Increasing bulb gross yield (t/ha^{-1}) with nitrogenous fertiliser whilst superficially helpful, does not necessarily lead to increased net yield (Marino et al. 2013). In Tasmanian onion production trials, experiments with various rates of N, P and K fertiliser concluded that top dressed N did increase bulb yield but also influenced the onion plants response to potassium (Laughlin 1989). These contrasting results and those from other studies (Costigan, Greenwood & McBurney 1983; Marino et al. 2013) demonstrate how genotype variation, soil type, nutrient status and latitude all affect crop growth and development in response to nutrient applications (Westerveld et al. 2003).

Nutritional guidelines typically used as a foundation for crop production are based on published recommendations sourced from controlled experiments in North America on Southport White Globe onions (Zink 1962, 1966), survey data from New South Wales, the Northern Territory Australia, and diagnostic records assimilated from laboratory analyses for bulb onions (Reuter & Robinson 1997). There are no complete data sets reflecting the nutritional requirements for intermediate day-length bulb onions, particularly those grown in the Southern Hemisphere for export markets. The motivation for this study was to expand the range of nutrient concentration data to facilitate plant tissue assessment of growing crops and to facilitate crop management for high net yield.

This study reports tissue nutrient concentrations for an intermediate daylength Creamgold onion in a cool-climate region across all key growth stages of commercial onion crops including harvested cured bulbs. We utilised this data in combination with soil physicochemical characteristics to assess the possible predictability of crop yield, skin disorders and bulb quality.

Materials and Methods

Field experiment

Thirty-four commercial onion crops in northern Tasmania, Australia, were monitored during the 2012/13 growing season. Crops were located within a 50 km radius of Longford (41°35'45.30"S 147° 7'18.35"E) and individual field soil types were initially identified using the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Australian Soil Map (C.S.I.R.O. 2014). These were confirmed through ground-truthing using the Australian Soil Classification (C.S.I.R.O. 2014) system. At each location, no allium crops had been grown for a minimum of five years prior. Within each crop, a representative area 50 m x 50 m, termed 'site,' was marked out prior to any cultivation and located to avoid irrigator tracks and spray runs.

Soil samples and fertiliser applications

From each site, 25 soil cores (20 millimetres diameter to a depth of 150mm) were taken in a 10-metre grid pattern using a stainless-steel corer and bulked. Soil samples were kept cool (<6°C) prior to dispatch overnight to Phosyn Analytical (Burleigh Heads, Queensland, Australia) where a subsample was taken for chemical analysis (Table 1).

Table 1. Soil physicochemical characteristics and element concentrations for the seven different soil groups surveyed. All values are means (*n* = number of commercial crops) and units are parts per million except for pH, Organic Matter (OM) and CEC. Individual field soil types were initially identified using the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Australian Soil Map (C.S.I.R.O. 2014). These were confirmed through ground-truthing using the Australian Soil Classification (C.S.I.R.O. 2014) system.

Soil Type	***n***	**pH H_2O**	*SEM*	**NO_3^-**	*SEM*	**P Olsen**	*SEM*	**S**	*SEM*	**Mn**	*SEM*	**B**	*SEM*	**Cu**	*SEM*	**Fe**	*SEM*	**Zn**	*SEM*
Dermosol	20	6.4	*0.1*	26.3	*5.5*	32.3	*2.9*	17.9	*1.2*	75.0	*9.0*	1.1	*0.1*	1.3	*0.1*	35.7	*3.0*	1.1	*0.1*
Ferrosol	6	6.4	*0.2*	16.3	*3.9*	50.5	*5.5*	14.2	*1.6*	39.1	*18.7*	0.8	*0.1*	1.2	*0.1*	25.3	*1.3*	1.5	*0.1*
Chromosol	3	6.6	*0.0*	7.5	*2.9*	51.0	*15.0*	8.7	*1.5*	39.3	*29.3*	0.6	*0.2*	0.7	*0.3*	68.7	*25.2*	1.4	*0.3*
Brown Kandosol	2	6.6		11.1		49.5		16.5		77.5		0.8		1.2		34.5		1.4	
Grey Vertosol	1	6.4		2.2		57.0		7.0		22.0		0.7		0.4		62.0		1.1	
Kurosol	1	5.8		25.0		51.0		14.0		14.2		0.6		0.4		67.0		0.9	
Tenosol	1	6.0		1.6		45.0		5.0		12.4		0.3		0.1		58.0		1.3	

Soil Type	***n***	**Cl**	*SEM*	**K**	*SEM*	**Mg**	*SEM*	**Ca**	*SEM*	**Na**	*SEM*	**Al**	*SEM*	**OM %**	*SEM*	**CEC (meq/100g^{-1})**	*SEM*
Dermosol	20	42.2	*8.9*	234.1	*31.2*	235.8	*23.5*	2101.2	*121.8*	70.1	*10.7*	19.9	*3.2*	6.6	*0.5*	13.6	*0.7*
Ferrosol	6	28.8	*2.6*	424.2	*80.2*	244.7	*56.2*	2294.3	*226.7*	64.8	*14.9*	19.7	*3.0*	11.5	*1.8*	15.1	*1.5*
Chromosol	3	13.3	*7.4*	144.7	*37.6*	90.7	*11.6*	1298.7	*255.2*	29.0	*8.0*	9.3	*3.5*	3.1	*0.6*	7.8	*1.5*
Brown Kandosol	2	27.5		268.0		173.0		1964.0		33.5		12.5		4.4		12.2	
Grey Vertosol	1	24.0		176.0		53.0		1038.0		18.0		8.0		2.2		6.3	
Kurosol	1	23.0		129.0		138.0		1416.0		41.0		64.0		6.5		9.4	
Tenosol	1	18.0		94.0		34.0		784.0		ND		5.0		1.8		4.6	

Total fertiliser application rates at crop establishment differed between plantings in autumn and those at the end of winter/spring according to commercial practice. Northern Tasmania has a high winter rainfall (B.O.M. 2014) and minimising the application of nitrogen to autumn-planted crops lessens the environmental loss. Hence, low analysis fertilisers were used in the autumn period and subsequently in comparison to winter/spring plantings had a higher sulphur concentration (single superphosphate has 11% sulphur) as a base component. Onion paddocks sown in autumn (May) had the following fertiliser incorporated prior to drilling; P at 70 kg/ha^{-1}, K at 80 kg/ha^{-1} and S at 90 kg/ha^{-1} with a further N application at 18 kg/ha^{-1} and P at 40 kg/ha^{-1} (as mono ammonium phosphate; MAP) applied below the seed at planting. Crops sown in winter/spring (end July – September) had N applied at 40 kg/ha^{-1}, P at 63 kg/ha^{-1}, K at 76 kg/ha^{-1}, S at 4.5 kg/ha^{-1} incorporated prior to drilling and a further application of N at 18 kg/ha^{-1} and P at 40 kg/ha^{-1} (MAP) applied during seed drilling.

Fertiliser applied during crop growth for autumn sown (May) onions had N at 115 kg/ha^{-1} as Urea and 135 kg/ha^{-1} as muriate of potash (MOP) broadcast in three equal amounts prior to rainfall or irrigation at 2TL, 5TL to just prior to bulbing. Crops sown in winter/spring (end July – September) had N at 80 kg/ha^{-1} as Urea and 95 kg/ha^{-1} of K as MOP applied in two equal amounts prior to rainfall or irrigation at 4TL and just prior to bulbing.

Sample analysis

All nutrient analyses were conducted by Phosyn Analytical according to established protocols following Rayment and Lyons (2011) and Kalra (1997) (Table 2).

Table 2. Summary of soil and plant nutrient properties measured and the methods used.

Soil Parameter	Soil Method	Reference
pH $CaCl_2$	4B2	(Rayment & Lyons 2011)
pH H_2O	4A1	
K, Mg, Ca & Na	15D3	
S	10B	
B	12C	
Al	15G1	
Cu, Fe, Mn & Zn	12A1	
EC	3A1	
Cl	5A2	
Organic Matter	6G1	
NO_3^--N	7B1	
P - Colwell	9B	
P – Olsen	9C	
CEC meq/100g^{-1} - Calculation	[K + Mg + Ca + Na + Al]	

Plant Parameter	Plant Method	Reference
P, K, Mg, B & Zn	ICP-AES	(Kalra 1997)
N & S	Dumas Combustion	
Chloride & NO_3-N	Colorometric	
Ca, Mn, Cu, Fe, Mo & Na	ICP-AES	

Onion planting

Commercial sowing of the trial and surrounding paddock used a three-bed precision drill to place onions in ten rows across each bed at 1.83m wheel centres with a consistent within row spacing between each onion seed longitudinally. This intra-row spacing varied from 47 to 80mm and was determined by customer orders for specific bulb sizes prior to planting. Cultivars sown in the survey area were six crops of Early Creamgold, twenty-three of Regular Creamgold, three of Hybrid Creamgold and two of Red onions. Within each 50 x 50m site an area of 22 x 25m, termed plot, was marked out avoiding all spray and irrigation runs. This site was used for all survey monitoring

and onion plant population was measured at between the first and second true leaf. The plant population ranged from 60 to 81 per m^2 (mean of 71 per m^2) in the season of study.

Crop monitoring

At weekly intervals, observations of leaf stage, plant vigour and leaf disease levels were used to measure crop development, while crop inputs, weed competition and soil moisture were also recorded to ascertain that crop management did not restrict crop growth. Irrigation requirement for all sites were calculated using the B.O.M. weather station records (Table 3) grower installed irrigation monitors, experiential knowledge and University of Tasmania crop water requirement tables (Agriculture).

Table 3. Rainfall record in millimetres by month for the onion growing and curing period May 2012 to April 2013 for the survey. Data were sourced from B.O.M. Strathbridge, Hagley Tasmania rainfall records (B.O.M. 2014)

2012								2013			
May	June	July	August	Sept	Oct	Nov	Dec	Jan	Feb	March	April
67	70	45	62	87	11	67	52	24	26	62	14

To assist in the objective rating of crop vigour and leaf disease pressure a seven-point scale was developed (Table 4). Growth stage was determined using the Brewster (2008) crop stage diagram.

Table 4**.** Seven-point rating scale used to describe crop vigour and leaf disease pressure. Assessment was undertaken by an experienced (>10 years) onion agronomist

	Rating - Crop vigour		**Rating - Leaf disease pressure**
1	plants stunted and/or yellowing	1	no disease evident / environmental conditions and seedling stage not suited to disease
2	small plants	2	no disease evident / small canopy, no closure / conditions make infection unlikely
3	below average growth	3	no disease evident / conditions suitable for infection
4	average growth	4	no disease evident / outbreak nearby
5	average to rapid growth	5	active mildew lesions visible on leaves
6	rapid plant growth and development	6	severe mildew – multiple leaves covered in spores
7	overgrowing / new leaves emerging together / loose neck	7	total leaf loss

Plant sampling and mature bulb assessment

Plants were randomly sampled (destructive) from plots at the 2, 4, 6, and 8 true leaf (TL) stages, at the commencement of bulbing, and at bulb harvest. Samples were taken before 11.30 am (Reuter and Robinson, 1997) on each sampling date, and were kept cool (<6^{o}C) until overnight shipment to the analytical laboratory (Phosyn Analytical, Burleigh Heads, Queensland, Australia). A minimum plant tissue sample of 200g was collected by placing a rod across a random section of the bed perpendicular to the planting rows and selecting those plants in each row closest to the rod. One hundred and fifty whole plants were collected over 15 beds at the 2 TL stage and 50 whole plants across 10 beds at the four true leaf growth stages by cutting at ground level with stainless steel scissors. At the six, eight true leaf and bulbing stages, the youngest fully expanded leaves (YFEL) from 50 plants were randomly harvested from across five onion beds.

During late bulbing, onion plants in all plots were lifted out of the ground by a tractor-drawn implement during the commercial crops harvest at the 80% “tops down” stage (Wright & Grant 1997). This implement windrowed the bulbs in the centre of the bed to cure *in-situ,* as is common practice in Tasmania. Curing times were between 14 – 21 days after lifting for May-June sown crops and 21 – 28 days after lifting for July-August sown crops. Tasmanian research has indicated a paddock curing time longer than thirty days encourages skin loss (Dennis et al. 2014). After curing, all bulbs were hand harvested and the dried foliage cut off with sterile secateurs at five cm above the bulb. Three netted bags, each comprising 10 to 11kg of bulbs between 40mm and 80mm in size were sampled from each plot. These were transported to a storeroom

and stored at 21°C and 60% RH from 6 am to 6 pm, and 11˚C and 80% RH from 6 pm to 6 am to mimic ambient temperatures but under controlled conditions.

Contingent on harvest time, bulbs were stored thirty to forty days post-harvest to finish curing before assessment commenced. Bulbs were subjected to simulated export handling using a tumbler adapted from Hole et al. (2002) and previously described by Gracie et al. (2006). In short, a 200-litre polypropylene drum fitted with an access port and two internal rubber strips was set at forty revolutions per minute for a period of ten minutes for Creamgold cultivars, and five minutes for red onion cultivars. Onions were able to move easily within the bag, and two bags were placed in the drum simultaneously. The tumbling method provides a consistent handling treatment across all crops and a relative estimate of a crops ability to withstand the rigours of packing and overseas shipping.

The measurement of bulb skin defects used was representative of export customer standards (Dennis et al. 2014) where any amount of visible bulb scale (flesh) is classified as a skin defect regardless of the cause (this includes splits, cracks or shelling). Samples were graded to this protocol, the rejected bulbs weighed and recorded, and the remaining samples returned to storage. Post initial assessment, one kilogram of onion bulbs from each site was randomly drawn from the bags then dispatched for nutrient analysis using the same tissue testing methodology as for field samples. The tumbling assessment was repeated at 90 days post-harvest.

Data analysis

Recursive partitioning analysis was used to assess the effects of plant tissue and soil nutrient levels on onion bulb quality and yield characteristics. This approach was selected due to some of the advantages over traditional parametric multivariate

analyses. This includes 1) the ease of interpretation, 2) the determination of thresholds of independent variables, and 3) that its use is not conditional on the assumption that explanatory variables must be independent of one another nor on the assumption that the level of observations must exceed the number of dependent variables (Hothorn, Hornik & Zeileis 2006). Recursive partitioning analyses were undertaken using “party” package and the ctree function in R version 3.4 (Hothorn, Hornik & Zeileis 2006). Models were also developed using proc GLMSELECT and proc GLM in SAS/STAT version 9.3 (SAS/STAT, SAS Institute, Cary, NC, USA). These models did not add further information to that provided by recursive partitioning and are not presented in the thesis.

Pearson coefficient matrix was used to assess associations among soil chemical properties and bulb nutrient levels at each stage of development. The quantiles for each soil and plant nutrient property were calculated using IBM SPSS statistics package version 24.

Results

Yield

The gross crop yield ranged from 53 to 96 t/ha^{-1} (mean of 75 t/ha^{-1}) in the season of study. This was consistent with long-term industry crop data for the production region of the 34 crops surveyed. The majority (73% *n*) of individual bulbs weighed between 96 to 126 grams.

Bulb tissue elemental content

By determining bulb dry matter and subsequent calculation of nutrient concentration allowed reporting of elements exported in kg/t of bulbs from the paddock (Table 5) (Bennett 1993).

Table 5. Nutrient recovery in kg/t of onions calculated on the mean plant tissue concentration and mean dry matter of bulbs, excluding onion foliage. Data are from the 34 sites surveyed.

Element	N	P	K	Ca	Mg	S
kg/t of bulbs	1.90	0.33	1.60	0.60	0.13	0.43

Plant tissue elemental content

The leaf and mature bulb nutrient tissue concentrations are provided alongside what we will refer to as the current recommendations as found in Reuter and Robertson (1997) (Table 6). Rather than categorising nutrients in two groups as either macro or micronutrients, this study grouped nutrients based on chemistry and function (Atwell 1999; Marschner 2011; Mengel et al. 2001). Nitrogen and sulphur are both Group 1 elements incorporated into the plant as oxyanions and covalently bound in reduced states, often within proteins (Marschener, 2011). The tissue concentrations of nitrogen

were higher than current recommendations at the 4TL, 6TL and mid-bulbing stages (Table 6).

Nitrate concentrations varied considerably during early crop development, approximately 80-fold at 2TL, however by mid bulbing the levels fell well below that currently recognised as a deficiency. Median concentrations of sulphur were consistently borderline deficient according to current standards before declining further in the mature bulb.

Phosphorus and boron are Group 2 elements that form covalent bonds in their fully oxidised states and while the role of phosphorus is broader than that of boron, both contribute to cell membrane integrity (Atwell 1999). Phosphorus tissue concentrations (median) at the 2TL stage were ca. 60% below that recommended while after establishment from 4TL to mid-bulbing these were within the range regarded as sufficient. At the mature bulb stage, the median phosphorus concentration of 0.25% was ca. 60% below the current upper recommendation level of 0.40% and below the current critical deficiency level of 0.30%. Comparing the published range of boron concentrations at various growth stages to this study showed plant tissue levels were sufficient.

Median concentrations of the ionic Group 3 elements calcium, magnesium and chloride were all below the accepted deficiency thresholds, and this was further pronounced during the later stages of crop development. For potassium, the recorded median concentrations were regarded as sufficient, except for the harvested bulb, in which the median tissue concentration was lower than the current recommendations. Sodium also an ionic element, fell below the limit of detection (<0.05ppm) for most crops and therefore, was not reported.

Group 4 nutrients are transition metals that generally function as constituents of metalloproteins; this group includes manganese, iron, cobalt, nickel, copper, zinc and molybdenum (Atwell 1999). The variation in tissue concentrations of Group 4 elements measured across the crops was many-fold. For example, molybdenum concentrations spanned 30-50-fold amongst crops, from just above the detectable limit at the 5th quantile of each growth stage up to 6TL, after which concentrations were over double the upper recommended levels at the 95th quantile. Iron, copper, zinc and manganese concentrations recorded at 2 and 4 TL growth stages varied approximately 3 to 10-fold between the 5th and 95th quantiles of the survey data. Further comparison of group 4 nutrient ranges is difficult due to limited published growth stage recommendations.

Table 6. Plant tissue concentrations:
Text in italics is the *reference range* for each nutrient taken from Reuter and Robinson (1997).
Data tabled below is 5th, median and 95th quantile range of nutrients from 34 crops functionally grouped in accordance with Atwell (1999).
TL denotes true leaf stage, WP denotes the whole plant sampled, YFEL denotes youngest fully emerged leaf and mature is cured bulb.
Key; trellis background denotes levels *below* reference range; vertical background denotes *above* the reference range, the horizontal background is *new* concentration data and no background is within the reference range.

Element	ppm/%	Quantile	2 TL/WP	4 TL/WP	6 TL/YFEL	8 TL/YFEL	Mid-Bulb/YFEL	Mature/Bulb
Nitrate	*ppm*	*Adequate*					*2000-4000*	
		Marginal					*<2000*	
	ppm	5th	**96.00**	**483.00**	**209.00**	**32.00**	**3.00**	**2.00**
		Median	**2900.50**	**2267.00**	**841.00**	**134.50**	**89.00**	**31.50**
		95th	**7489.00**	**3981.00**	**2533.00**	**648.00**	**726.00**	**91.00**
Group 1								
Nitrogen	*%*	*Adequate*	*4.57*	*3.63*	*2.60*	*2.50-3.50*	*1.50-1.75*	*1.20-1.35*
		Low					*1.30*	*1.00*
	%	5th	3.56	**3.89**	**3.34**	2.43	**2.32**	1.11
		Median	5.11	**4.71**	**3.89**	2.96	**2.84**	1.36
		95th	6.26	**5.34**	**4.59**	3.66	**3.73**	1.77
Sulphur	*%*	*Adequate*		*0.50-1.00*	*0.50-1.00*			
		Deficient		*0.30-0.49*	*0.30-0.49*			
	%	5th	**0.37**	0.33	0.33	**0.28**	**0.32**	**0.23**
		Median	**0.50**	0.48	0.48	**0.46**	**0.43**	**0.30**
		95th	**0.68**	0.64	0.62	**0.67**	**0.77**	**0.47**
Group 2								
Phosphorus	*%*	*Adequate*	*0.44*	*0.31*	*0.34*	*0.25-0.40*	*0.30-0.40*	*0.35-0.40*
		Deficient					*0.20*	*0.30*
	%	5th	**0.21**	0.22	0.27	0.26	0.21	**0.20**
		Median	**0.27**	0.30	0.32	0.32	0.27	**0.25**
		95th	**0.41**	0.36	0.41	0.38	0.36	**0.34**
Boron	*ppm*	*Adequate*		*22-60*	*30-45*		*25-40*	
		Deficient		*18-22*			*<20*	
	ppm	5th	**17.00**	17.00	16.00	**17.00**	17.00	**14.00**
		Median	**21.00**	25.00	21.50	**22.00**	26.00	**15.50**
		95th	**44.00**	37.00	43.00	**37.00**	37.00	**23.00**
Group 3								
Potassium	*%*	*Adequate*	*4.18*	*3.48*	*3.68*	*2.50-5.00*	*1.60-2.20*	*1.70-1.85*
		Deficient					*1.30*	*1.50*
	%	5th	1.85	2.43	1.39	1.09	1.20	**0.86**
		Median	4.78	4.53	3.17	2.24	2.01	**1.20**
		95th	7.92	6.58	4.29	3.02	2.98	**1.51**

Element	ppm/%	Quantile	2 TL/WP	4 TL/WP	6 TL/YFEL	8 TL/YFEL	Mid-Bulb/YFEL	Mature/Bulb
Group 3								
Calcium	*%*	*Adequate*	*1.60*	*1.28*	*1.28*	*1.50-3.50*	*2.20-2.90*	*0.40-0.50*
	%	5th	1.11	1.04	**0.71**	**0.58**	**0.60**	0.3
		Median	1.54	1.43	**0.95**	**0.78**	**1.11**	0.41
		95th	2.12	1.76	**1.27**	**0.97**	**1.51**	0.58
Magnesium	*%*	*Adequate*	*0.47*	*0.35*	*0.29*	*0.30-0.50*	*0.60-0.80*	*0.15-0.20*
		Deficient				*0.22-0.24*		
	%	5th	**0.21**	0.19	**0.16**	**0.12**	**0.14**	**0.08**
		Median	**0.29**	0.28	**0.21**	**0.17**	**0.21**	**0.10**
		95th	**0.47**	0.42	**0.28**	**0.26**	**0.33**	**0.13**
Chloride	*%*	*Adequate*					*4.00-5.00*	*0.32-0.42*
	%	5th	**0.75**	**0.90**	**0.59**	**0.52**	**0.38**	**0.19**
		Median	**1.17**	**1.33**	**0.91**	**0.63**	**0.70**	**0.27**
		95th	**1.93**	**1.82**	**1.62**	**1.00**	**1.29**	**0.36**
Group 4								
Manganese	*ppm*	*Adequate*		*50-250*	*40-100*		*55-65*	
		Deficient		*30-49*			*<40*	
	ppm	5th	**54.00**	50.00	36.00	**37.00**	40.00	**16.00**
		Median	**143.50**	101.50	74.00	**67.50**	91.00	**23.50**
		95th	**528.00**	170.00	148.00	**96.00**	186.00	**59.00**
Iron	*ppm*	*Adequate*			*60-300*			
	ppm	*Deficient*			*50-59*			
	ppm	5th	**82.00**	**68.00**	48.00	**33.00**	**32.00**	**35.00**
		Median	**167.00**	**120.00**	55.50	**44.50**	**45.00**	**43.00**
		95th	**1092.00**	**264.00**	83.00	**55.00**	**65.00**	**72.00**
Copper	*ppm*	*Adequate*			*6-25*		*5-10*	
		Deficient			*<5*		*<4*	
	ppm	5th	**1.00**	**1.80**	3.10	**2.30**	2.40	**1.30**
		Median	**6.10**	**6.50**	6.30	**6.65**	6.70	**3.60**
		95th	**9.70**	**9.90**	9.30	**27.80**	24.80	**5.10**
Zinc	*ppm*	*Adequate*			*20-55*			
	ppm	5th	**14.00**	**12.00**	14.00	**16.00**	**14.00**	**11.00**
		Median	**20.00**	**20.00**	25.00	**22.00**	**19.00**	**17.00**
		95th	**46.00**	**30.00**	38.00	**33.00**	**34.00**	**31.00**
Molybdenum	*ppm*	*Adequate*			*0.15-0.30*			
	ppm	5th	**0.02**	**0.02**	0.02	**0.02**	**0.02**	**0.01**
		Median	**0.23**	**0.21**	0.13	**0.11**	**0.09**	**0.07**
		95th	**1.02**	**0.64**	0.62	**0.36**	**0.38**	**0.31**

Soil to plant tissue correlation

A correlation matrix of the available soil elements to plant tissue concentrations demonstrated a low level of covariation (Table 7). Exceptions to this were magnesium and manganese ($p<0.001$), which showed a positive association over the majority of growth stages. Positive correlations were apparent at the 6 to 8 true leaf (TL) growth stage of onions for chloride ($p<0.001$), 8 TL for iron ($p<0.001$) and bulbing for iron (p0.05) but no other correlation was apparent. Two TL growth stage and mature bulb plant tissue concentrations of copper ($p<0.001$) showed a positive correlation.

Table 7. Correlation matrix of soil and plant tissue nutrient at 2, 4, 6 & 8 true leaf (TL), and mid and mature harvested bulb. Plant tissue component measured were either whole plant (WP), youngest fully emerged leaf (YFEL) and or entire cured bulb (Bulb). Pearson's Correlation Coefficient (r), *p-value* < 0.05 bolded, *n*=34.

	Plant Growth Stage	2 TL/WP	4 TL/WP	6 TL/YFEL	8 TL/YFEL	Mid-Bulb/YFEL	Mature/Bulb
Soil Concentration							
Nitrate	r	-0.003	0.025	0.132	-0.211	-0.105	0.064
	p-value	0.987	0.894	0.473	0.245	0.566	0.726
Sulphur	r	0.377	0.254	0.117	0.140	0.378	0.380
	p-value	0.028	0.148	0.509	0.431	0.028	0.027
Phosphorus	r	0.394	-0.174	0.113	0.169	0.164	-0.035
	p-value	0.021	0.325	0.525	0.341	0.354	0.845
Boron	r	-0.182	-0.247	-0.224	-0.229	-0.163	-0.141
	p-value	0.302	0.160	0.203	0.194	0.357	0.426
Potassium	r	0.159	-0.016	0.143	0.232	0.073	0.122
	p-value	0.370	0.929	0.420	0.187	0.683	0.493
Calcium	r	-0.058	0.006	-0.065	-0.168	0.237	-0.281
	p-value	0.745	0.972	0.716	0.342	0.178	0.108
Magnesium	r	***0.458***	***0.681***	***0.687***	***0.648***	***0.640***	***0.476***
	p-value	***<0.01***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.01***
Chloride	r	-0.039	0.427	***0.579***	***0.463***	0.075	0.154
	p-value	0.831	0.013	***<0.001***	***<0.01***	0.679	0.391
Manganese	r	***0.468***	***0.619***	***0.551***	***0.546***	***0.474***	0.105
	p-value	***<0.01***	***<0.001***	***<0.001***	***<0.001***	***<0.01***	0.5554
Iron	r	-0.186	-0.245	0.182	***0.542***	***0.338***	-0.289
	p-value	0.293	0.162	0.303	***<0.001***	***0.05***	0.097
Copper	r	0.278	***0.660***	0.107	0.301	0.112	***0.574***
	p-value	0.111	***<0.001***	0.549	0.084	0.528	***<0.001***
Zinc	r	0.223	0.122	0.235	0.169	0.258	0.052
	p-value	0.205	0.492	0.181	0.340	0.141	0.772

Bulb nutrient concentration and leaf disease influence skin quality

Downy mildew (*Peronospora destructor*) disease observations recorded during the mid bulbing growth stage were associated with bulb skin loss. Of the 34 crops, the first division in the binary recursive partitioning tree grouped 25 crops (node 2) together, these associated with disease observation scores ≤3 (Figure 2) and a lower probability of skin loss. The remaining 9 crops were concomitant with disease observation scores >3 (node 3) and a higher predicted probability of skin loss. Across the terminal nodes, the predicted median skin loss increased from 18% at node 2 to 25% at node 3.

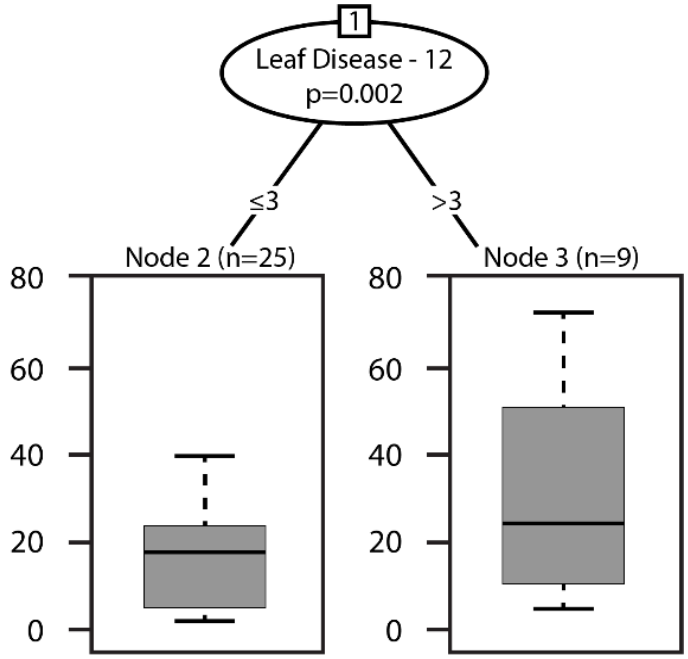


Figure 2. Conditional reference regression tree showing the relationships between percentage onion bulbs with skin loss and potential predictor variables. All potential plants and soil variables were evaluated. The cut points for each variable are indicated after each node. The numbers within squares indicate node labels. The mean skin loss (%) are presented as box plots (median values and interquartile ranges) within the terminal nodes. Leaf disease 12 = bulbing growth stage downy mildew disease observation from Table 4.

Bulb nutrient concentration and skin disorders of bulbs

Bulb moisture percentage and bulb molybdenum concentrations were also associated with the severity of skin loss. The first cut-off grouped 27 crops (node 2) together, these having a bulb moisture ≤87.6% (Figure 3) and a lower probability of skin loss.

This node (Node 2) was further partitioned into two groups, 7 crops having molybdenum tissue concentrations ≤0.047ppm (node 3) and 20 with molybdenum concentrations above this level (node 4). The predicted probability of skin loss increased from 4% at node 3 to 47% at node 5, the remaining 7 crops in this latter node associated with bulb moisture >87.6% (node 5) and with no association with molybdenum.

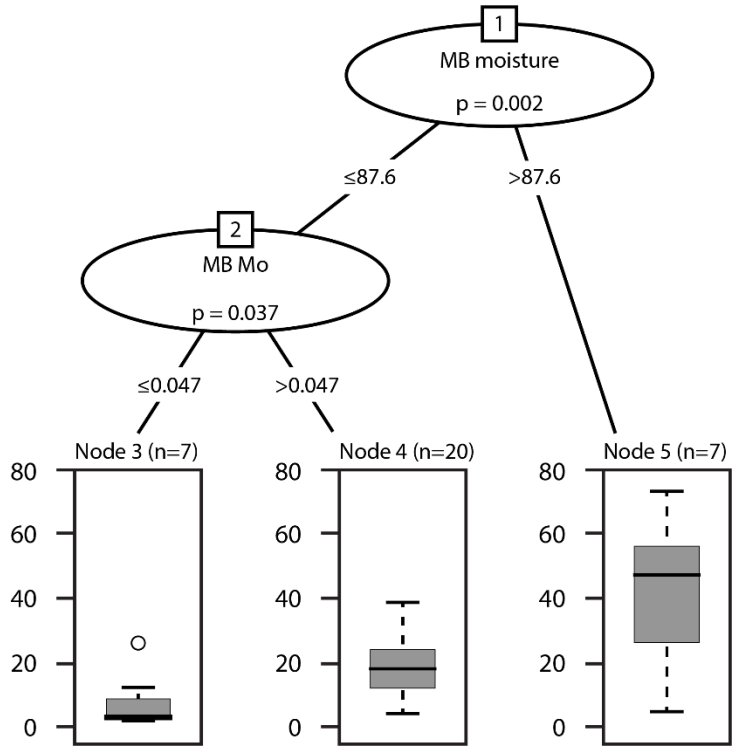


Figure 3. Conditional reference regression tree showing the relationships between percentage onion bulbs with skin loss and potential predictor variables. All potential plants and soil variables were evaluated. The p-value within each node represents the significance level of each split. The cut points for each variable are indicated after each node. The numbers within squares indicate node labels. The mean skin loss (%) are presented as box plots (median values and interquartile ranges) within the terminal nodes. Open circles indicate outliers. MB moisture = mature bulb moisture percentage, and MB Mo = mature bulb molybdenum concentration (ppm).

Leaf tissue sulphur concentrations measured at mid-bulbing were linked to skin loss. The split of sites grouped 27 crops (node 2) together, these having leaf sulphur

concentrations ≤0.6% (Figure 4). Comparing the 2nd and 3rd terminal nodes, the predicted probability of skin loss increased from 16% to 39%.

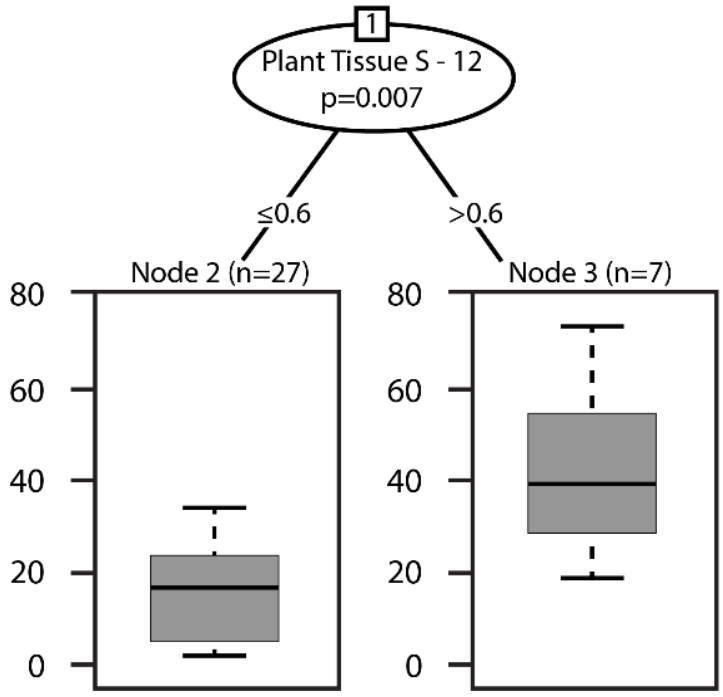


Figure 4. Conditional reference regression tree showing the relationships between percentage onion bulbs with skin loss and potential predictor variables. All potential plants and soil variables were evaluated. The cut points for each variable are indicated after each node. The numbers within squares indicate node labels. The mean skin loss (%) are presented as box plots (median values and interquartile ranges) within the terminal nodes. Open circles indicate outliers. Plant S 12 = bulbing growth stage, sulphur concentration (%).

Sulphur tissue concentrations at bulbing were also associated with the severity of skin loss, but the influence of this predictor was less important than nitrate tissue concentrations. Of the 34 crops, the first split in the binary recursive partitioning tree grouped 10 crops (node 2) together, these being associated with bulb nitrate concentrations less than 20ppm (Figure 5). The remaining 24 crops (node 3) were again split into two groups, 16 associated with sulphur concentrations ≤0.34% (node 4) and 8 with sulphur concentrations above this cut-off (node 5). Across the terminal

nodes, the predicted probability of skin loss increased from 9% node 2 to 39% node 5.

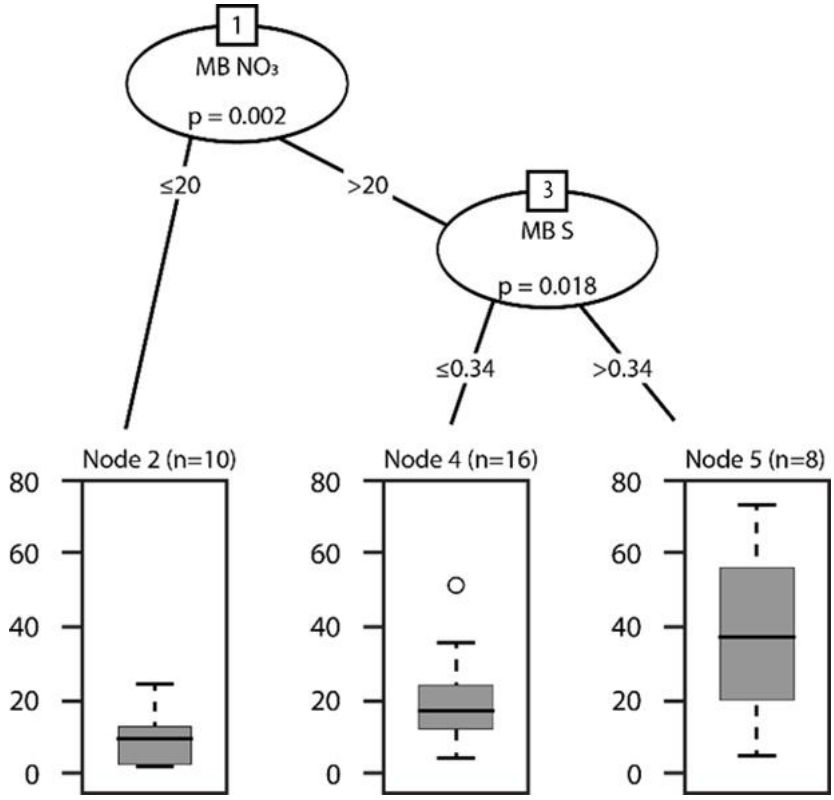


Figure 5. Conditional reference regression tree showing the relationships between percentage onion bulbs with skin loss and potential predictor variables. All potential plants and soil variables were evaluated. The p-value within each node represents the significance level of each split. The cut points for each variable are indicated after each node. The numbers within squares indicate node labels. The mean skin loss (%) are presented as box plots (median values and interquartile ranges) within the terminal nodes. Open circles indicate outliers. MB NO_3^- = mature bulb nitrate concentration (ppm), and MB S = mature bulb sulphur concentration (%).
Nitrate concentration in mature bulbs (MB) with steps of sulphur concentration indicating skin loss prediction. Increased skin loss ($p<0.01$) was recorded at mature bulb NO_3^- concentrations of >20 ppm. Adding a second step at bulb S concentrations >0.34% further increased skin loss ($p<0.05$).

Discussion

Nutritional programs for onions have been successfully developed based on a direct relationship between yield and crop nutrient removal (Bennett 1993), yet the less direct linkages between crop nutrition and temporally separated outcomes such as storage life and quality defects are more difficult to detect. In this study, many nutrient tissue concentrations recorded for commercial onion crops in Tasmania, Australia, varied from published recommendations for healthy crop growth and development. This work has provided new and additional information on crop nutrition requirements across key growth stages.

Across a number of key growth stages slightly higher nitrogen levels were recorded in this survey against the current recommendation (Reuter & Robinson 1997). This widely cited reference is based on a different cultivar, soil type and N application timing than the methodology used for this study (Zink 1962, 1966). These factors are likely responsible for the different tissue concentrations observed. This is not unprecedented as Westerveld et al. (2003) described varying nitrogen tissue concentrations due to soil type, production practices, climate and cultivar. The decline in recorded nitrogen concentrations during crop growth and development almost certainly reflects the nitrogen fertilisation regime in Tasmania, with the application of all nitrogenous fertiliser ceasing well prior to the commencement of bulbing. Thus, it was anticipated that nitrogen levels would decline towards bulbing. This approach is also practised in New Zealand (Wright, 1993) with similar cultivars. With this management, crops exhibit symptoms of nitrogen limitation at bulbing observed as slight canopy yellowing from the centre of the bed outwards just prior to top collapse (Rabinowitch & Brewster 1990).

Linked closely with total nitrogen is plant concentrations of nitrate. As a mobile ion in the soil solution nitrate is rapidly acquired by plants and if not taken up by the root system is readily leached from the soil profile (Baldwin 2009). Plant nitrate averaged 89 ppm at mid-bulbing in this study compared with 2000-4000 ppm published for unspecified cultivars of onion bulbs in Romania (Davidescu & Davidescu 1982). Importantly, the Romanian study used sap analysis that reports different results to the dry ash analysis used in this study. Testing nitrate by sap analysis is an indication of mobile plant nitrogen at a specific time. As with all elements, establishing realistic levels for onions would require individual area and crop-specific levels to be established for interpretation and prescriptive application (Westerveld et al. 2003).

Further evidence supporting prescriptive application is provided by the concentrations recorded for the Group Three ionic element (Atwell 1999), all of which were lower across growth stages when compared with published levels. The lack of availability of both cations and anions in the soil solution may be in part responsible for these observations, although consistent covariance across growth stages between soil availability and plant tissue concentrations were only observed for magnesium, fourth in the lyotropic series. Magnesium tissue concentrations were low in five of six recorded growth stages when compared to current recommendations. Magnesium application has previously been linked to yield increases in onions (Kleiber, Golcz & Krzesinski 2012) however the plant tissue ranges in this study are consistent with wider research, and higher than that observed in this study (Boyhan & Kelley 2007). Although onions do not exhibit classic magnesium deficiency symptoms (Bennett 1993) no relationship was observed between magnesium and bulb quality parameters. The difficulty in relating ion availability in the soil solution is not surprising given the complexity of chemical interactions occurring between the soil solution and the root

system. Additionally, there is considerable evidence that the ions moving within the plant are not necessarily related to the concentrations accumulated in root tissue (Fried & Shapiro 1961), hence shoot tissue concentrations do not necessarily reflect availability to the root system. Results presented (Table 7) support this conclusion indicating little correlation, generally, between the majority of soil and plant concentrations (Marschner 2011).

Calcium, also Group 3, plays a multifunctional role in plant tissues and the tensile strength of skins has been posited as resulting from calcium cross-linkages with pectic carbohydrates (Ng et al. 2000). While this element has also been positively linked to onion bulb scale firmness (Coolong & Randle 2008) deficiencies are rarely seen in the field (Bennett 1993). In this study, the median Ca tissue concentrations from 6TL to mid-bulbing was lower than recommended by Reuter and Robinson (1997) but were consistent with the critical ranges published in the *Onion Production Guide* (Boyhan & Kelley 2007) and experiments with onions cultivated in sand (Pankov 1984). One earlier study of RCG and ECG onions identified leaves five to seven as the primary source of the first intact skin of these cultivar's bulbs (Gracie et al. 2012). Given that low calcium tissue concentrations were beyond the time at which these leaves were developing, it is unlikely to have influenced skin quality, as also evidenced by the lack of any correlation between skin loss and tissue calcium levels in this study. We therefore propose that the tissue calcium levels in this study were sufficient.

Crop yields were comparatively high as evidenced by the gross crop yield range of 53 to 96 t/ha^{-1} (mean of 75 t/ha^{-1}) (F.A.O.). There were no correlations between tissue nutrient concentrations and gross yields suggesting that nutrient availability was not a primary factor in yield determination. Although no direct relationships were established with yield, this study has shown that within the nutritional range reported for these

crops, increasing amounts of skin loss could be linked to nutrition. Specifically, levels of nitrate, sulphur, molybdenum and bulb moisture were associated with increased bulb skin loss.

The assimilation of nitrogen in plants via the conversion of nitrate to ammonium is mediated by the bio-chemical pathways catalysed by nitrate reductase. All the elements linked to increased skin loss are also involved in nitrate reduction. While also involved in sulphur metabolism (Hänsch & Mendel 2009) molybdenum's incorporation as a complex in the nitrate reductase dimer is one of the key roles played by this element in plant metabolism (Taiz & Zeiger 2010). Similarly, a sulphur-iron cluster is a prosthetic group of the enzyme nitrite reductase, this enzyme facilitating the conversion of nitrite to ammonium (Taiz & Zeiger 2010). Reductively, we postulate that as nitrate levels increased in the plant tissue, the quantities of both molybdenum and sulphur required for its metabolism may have also increased in concert. Increased nitrate concentrations would have facilitated growth rate, possibly through increased cell volume and a subsequent increase in bulb moisture content. Hence, the increase in skin loss associated with these elements and bulb moisture may reflect increased growth rates resulting in increased circumferential tension and a resultant failure in skin tissues.

Downy mildew infection (*Peronospora destructor*) contributing to skin loss has been reported in Tasmania (Gracie et al. 2006) as has the contribution of nitrogen application to an increase in the severity of this disease (Develash & Sugha 1997). In the absence of any mechanistic data indicating how nitrogen facilitates increases infection, we conclude that increased nitrogen availability and subsequent heavier canopies, may have improved the suitability of the canopy microclimate for infection.

The increased level of infection levels highlights the intricacies involved in nitrogen management during crop development.

This study has highlighted that the complexity of onion crop production combined with varying cultivar selection requires prescriptive application data for bulb onions (Boyhan et al. 2014). Plant concentrations varied widely in the dynamic growing environment and element interactions played a significant role in the quality outcome of the harvested crop. This point is illustrated by the marketable onion yield following the handling assessment, where fourteen crops (41% *n*) were flagged as unsuitable for long transit export. Tissue concentrations for each element were defined against key growth stages and quantile trend lines are reported to provide a clearer assessment of crop nutrition and deficiencies. Plant analysis based on prescriptive data can assist in diagnosing nutritional problems or potential problems in the crop. Plant analysis results also have an application into managing fertiliser amendments of the same crop grown in subsequent seasons including increased or decreased rates based on tissue test results (Hochmuth et al. 2010). This study has defined some elements that have not had a comprehensive survey reported by growth stage previously in Australia or linked nutrition to quality outcomes, rather than just gross yield.

Does manipulating nutrient levels of onion plants affect skin and bulb quality?

Abstract

The Tasmanian onion industry is underpinned by the production and supply of high-quality onion bulbs that can withstand the rigours of handling, shipping and repacking upon arrival at key export destinations and have long-term storage characteristics. Despite this reputation, some shipments of onions lose quality and anecdotally these poor-quality traits are partially attributable to crop nutrition. At present, little is understood about the nutritional effect on onion bulb quality in this commercial context.

This chapter expands on the associations recorded between plant tissue concentrations of nitrate, sulphur and molybdenum and elevated levels of bulb skin loss. Experiments involving the three nutrients were undertaken in four commercial crops of open-pollinated Creamgold cultivars. Amending the base fertiliser program with additional sulphur, molybdenum or nitrogen led to higher plant tissue concentrations of that nutrient, however, these elevated tissue concentration levels did not significantly affect the incidence of skin loss nor long-term bulb storage characteristics. Higher applications of sulphur resulted in higher pyruvate levels in mature bulb concentrations. Molybdenum application increased plant Mo concentrations alone, although when combined with sulphur application the response was reduced. Application of ammonium sulphate increased both plant nitrogen concentrations across all growth stages and nitrate levels across the various growth stages and decreased soluble solids in mature bulbs. Commercial production site had the greatest influence on bulb storage attributes and incidence of skin loss.

Introduction

Onion bulbs, post machine harvest, typically have between one to three skins depending on genetic potential and crop growing conditions (Brewster 2008; Hole, Drew & Gray 2002). Bulbs that do not have an entire intact skin at either of the local factory or export destination packers are deemed commercially unacceptable and sent to waste (Wright & Grant 1997). It is critical, therefore, that onions at harvest maintain multiple skin layers that are not easily dislodged to allow for the likelihood of damage or loss of layers during handling and storage. Whilst solving skin quality issues through breeding is an option, crops of the same genotype vary in skin quality indicating that agronomic practices and environmental conditions play a key role (Ariyama et al. 2006; Costigan, Greenwood & McBurney 1983). Understanding the effect growing conditions and nutrition have on bulbs is important for consistent consumer quality, maximising returns and long-term bulb storage life (Boyhan, Torrance & Hill 2007; Boyhan et al. 2014).

The susceptibility of a bulb to lose skins during storage is also a function of the inherent material and structural properties of the skin as determined during onion plants growth and development (Hole, Drew & Gray 2002). In Tasmanian crops, the first skin to envelop the entirety of the bulb (entire skin) is commonly derived from leaves 5 to 8 for the variety “Early Creamgold” (ECG), and from leaves 6 to 8 for “Regular Creamgold” (RCG) (Gracie et al. 2012). Prior to bulb initiation, an onion plant continually produces leaves bearing laminas (blades). At the onset of bulbing leaf blade formation ceases and bladeless sheaths are produced. Both types of leaf sheaths increase in thickness via cell enlargement causing the bulb to swell. During bulbing and after the production of the bladeless sheaths, the meristem then produces bladed leaf initials that will later elongate during sprouting. The bulb, therefore,

consists of three distinct types of leaves produced in a defined sequence. (Abdalla & Mann 1963). It is likely that circumferential tension on the outer bulb surface is created by the expansion of these tissues. During the maturation process, this circumferential tension and desiccation of the outer leaf sheaths likely contribute to the formation of the thin dry skins that encase the bulb.

The interaction between skin material and structural properties with moisture content play a significant role in determining skin retention. An important structural property contributing to skin strength is the thickness of the compressed scale. This compressed scale is comprised of multiple layers of desiccated cellular tissue interspersed with vascular traces. Recent research indicated that the outer scale desiccates from the inside out. This suggested that cell death in the outer scales, during skin formation, is an internal structured process. This process not only from air desiccation but also programmed cell death, gradually decreased toward the inner scales (Galsurker et al. 2016). Thicker skins are derived from higher cross-sectional cell numbers and have a higher resistance to mechanical failure (Gracie et al. 2012). Onion skins are reported to range in thickness from 0.02 to 0.17 mm (Gracie et al. 2012; Hole, Drew & Gray 2002) with the final thickness of skins as a function of the structural material in the cross-sectional tissue and moisture content (Hole, Drew & Gray 2002). The moisture content of onions skins varies relative to their surroundings with bulbs losing moisture through skins rather than base plate or neck. Skin flexibility and splitting can vary with different atmospheric conditions (Brewster 2008).

Skin quality can be compromised if the onions are handled roughly at either harvest or initial grading (Gracie et al. 2012). Cracks appearing around the circumference of bulbs typically indicate damage from dropping (Brewster 2008) which can occur at one or several instances on removal from the store, grading, arrival unloading or repacking

(Gracie et al. 2012). Creamgold bulbs grown for storage in South Australia showed increased skin fragility when linked to variable fertiliser applications (Maier, Dahlenburg & Twigden 1990b). Together suggest nutrient application, particularly during early growth, could increase skin thickness. The intact skin protects the inner flesh from damage and assists with dehydration loss in the storage process. The loss of onion skins in storage can double bulb weight loss due to desiccation and this action promoted early sprouting (Apeland 1969).

Onion cultivars that mature at longer daylength generally form dormant bulbs that are naturally suitable for long-term storage (Petropoulos, Ntatsi & Ferreira 2016). Bulbs that break dormancy and then sprout in storage are a major problem when shipping to export destinations. This can be managed by in-crop use of sprout retardants such as maleic hydrazide, although these chemicals are negatively perceived in export markets (Suojala, Salo & Pessala 1998). Storage life and quality of onion bulbs are known to be adversely affected by excessive use of nitrogen before harvesting as this delays maturation and results in higher bulb moisture loss during storage (Petropoulos, Ntatsi & Ferreira 2016). Reducing nitrogen quantities can increase the time before sprouting occurs during storage (Sørensen & Grevsen 2001). In addition to shortening storage life, expansion of the base plate during sprouting also contributes to skin losses and quality problems (Brewster 2008).

Quality improvements in onion crops have been linked with remedial applications of crop nutrient. Supplementing crops with calcium chloride ($CaCl_2$) showed improved onion bulb firmness at harvest on soils containing low available calcium (Coolong & Randle 2008). Calcium and ammonium applications were shown to increase onion bulb weights linked to deposits of carbonaceous compounds (Feagley & Fenn 1998). However, the weight of the entire plant did not increase indicating disproportional

sectioning of the compounds. Increased N rates positively correlated with bulb losses in long-term ambient air storage (Rabinowitch & Currah 2002). Increased chlorophyll content was noted with zinc application, the Zn acting as a structural and catalytic component of plant proteins and enzymes (Almendros et al. 2015).

Nutrient concentration and skin loss reported in the previous chapter identified positive associations between bulb tissue concentrations of sulphur, molybdenum and nitrate and bulb quality. This chapter explored the potential causal nature of these associations.

Materials and Methods

Field experiment

A 50 x 50 m area within four separate commercial onion crops was established for the experiments. All areas were sown during the 2013/14 growing season and located within a 25 km radius of Longford, Tasmania (41°35'45.30"S, 147°7'18.35"E). Each site represented a separate common soil type (Table 8) in the region and were initially identified using the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Australian Soil Map (C.S.I.R.O. 2014). These were confirmed through ground-truthing using the Australian Soil Classification (C.S.I.R.O. 2014) system, on-site inspection and laboratory analysis of type.

Two experiments were undertaken in the identified area in each the four sites: Experiment 1) a two by two factorial in an RCBD comprising of sulphur and molybdenum, and Experiment 2) a single factor experiment in an RCBD comprising of ammonium sulphate. The two were integrated and shared the untreated control; with four replicates, the area comprised twenty plots in total.

In the two by two factorial experiment sulphur was applied as sulphur trioxide (Brimstone 90, NEAIS) at a rate of 90 kg/ha^{-1} using a hand spreader. Molybdenum (Sodium Molybdate, Barmac) was applied at a rate of 780 g/ha^{-1} using a hand sprayer. Both were applied at 1 ½ true leaves. The single factor experiment of ammonium sulphate was applied at a total elemental quantity of N at 94 kg/ha^{-1} and S at 108 kg/ha^{-1}. This was split-applied in three even quantities over the growing crop at 2, 4 and 8 true leaf growth stages.

Each plot comprised three adjacent 1.83 x 12 m beds located in a representative crop area away from boom sprayer and irrigator tracks. Agronomy for all sites was consistent with standard commercial practice.

Soil sample and base fertiliser applications

The soil type and chemical properties at each site are provided (Table 8. The soil sampling and analysis protocol were consistent with the methods described in Chapter two.

Table 8. Soil chemical properties and element concentrations at each of the four different sites. All units are parts per million except for pH, Organic Matter (OM) and CEC. Individual field soil types were initially identified using the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Australian Soil Map (C.S.I.R.O. 2014). These were confirmed through ground-truthing using the Australian Soil Classification (C.S.I.R.O. 2014) system.

Site	**ECG10**	**ECG20**	**RCG50**	**RCG60**
Soil Classification	**Yellow Dermosol**	**Brown Kandosol**	**Red Ferrosol**	**Chromosol**
pH-H_2O	6.3	6.5	6.6	5.8
NO_3-ppm	2.2	20.1	41.6	29.2
Olsen P-ppm	60	44	58	41
K-ppm	386	289	593	230
Ca-ppm	1706	2434	3226	928
Mg-ppm	182	208	486	158
S-ppm	14	19	29	16
Mn-ppm	80	58	111	10
B-ppm	0.7	0.8	2.1	0.7
Cu-ppm	1.4	0.8	1.5	0.4
Fe-ppm	39	39	23	176
Zn-ppm	1.3	1	2.1	0.6
Cl-ppm	31	66	151	78
Na-ppm	35	39	99	113
Al-ppm	17	23	24	29
OM %	8.2	6.4	5.7	4.1
CEC (meq $100g^{-1}$)	22.4	15.1	11.4	7.4

Pre-drilling fertiliser application rates were divided into two distinct groupings of autumn and spring plantings (Table 9). Northern Tasmania has a high winter rainfall and minimising the application of N to autumn sown crops lessens the environmental loss. The use of low analysis fertiliser for crop requirements in this planting period also has significant S content (SSP with 11% S) as a base component.

Table 9. Fertiliser amendments applied to the four sites: sites ECG10 and ECG 20 were sown in May and sites RCG50 and RCG60 were sown in September.

		ECG10				ECG20				RCG50				RCG60			
TL Stage	**Unit**	**N**	**P**	**K**	**S**	**N**	**P**	**K**	**S**	**N**	**P**	**K**	**S**	**N**	**P**	**K**	**S**
Pre-Plant	kg/ha^{-1}	-	70	80	90	-	70	80	90	40	63	76	4.5	40	63	76	4.5
Drilling	kg/ha^{-1}	18	40			18	40			18	40			18	40		
2	kg/ha^{-1}	39		42		46		50		-		-		-		-	
4	kg/ha^{-1}	46		50		46		50		41		45		32		35	
8	kg/ha^{-1}	41		45		46		50		32		35		37		40	

Seed were sown to 1.83m wheel centre beds using precision drills so that each bed comprised 10 rows. A target intra-row spacing of 47mm for May sown crops of cv. Early Creamgold (ECG) and 70mm for September sown crops of cv. Regular Creamgold (RCG). Germination, field factor and vigour results for each seed lot were used as part of the of seed rate calculation. Testing of seed quality was conducted by seedPurity Pty Ltd, Margate Tasmania and Botrytis seed borne assessment by Peracto Pty Ltd, Devonport Tasmania. Although Botrytis levels were reported to be nil for both internal and external seed assessment, a standard commercial protocol of seed treatment with two fungicides was applied, followed by two separate foliar in-crop applications of Filan™ (500 g/kg Boscalid).

One 1.83m bed width was left between blocks to minimise any cross-contamination from the four treatments and five replicates. At the one true leaf (TL) stage all plots were counted to establish plant population. This measurement was taken using a 1.83m by 0.55m internal area quadrat placed across the bed from both wheel centres to give a true one-m^2 measurement.

Crop monitoring and plant sampling

For each plot, plant development was recorded and plant tissue sampling for nutrient analysis was undertaken as described in Chapter 2. In summary, plants were randomly sampled from plots at the 2, 4, 6, and 8 true leaf (TL) stages, at the commencement of bulbing, and at bulb harvest. A minimum plant tissue sample of 200g in weight was collected. This comprised one hundred and fifty plant shoots at the 2 TL stage and fifty at the 4 TL stage. At the 6 TL, 8 TL and bulbing stages the youngest fully expanded leaves (YFEL) were used. Post machine lifting, field curing times for bulbs between 14 – 21 days for May-June sown crops and 21 – 28 days July-August

sown crops were observed. After curing all bulbs were hand harvested and the desiccated foliage severed at 5cm above the bulb.

Growth and harvest

Experiments were conducted within commercial onion crops that had a weekly agronomic assessment. Irrigation requirement for all sites were calculated using the B.O.M. weather station records (Table 10) grower installed irrigation monitors, experiential knowledge and University of Tasmania crop water requirements literature (Agriculture).

Table 10. Rainfall record in millimetres by month for the onion growing and curing period May 2013 to April 2014 for Experiments 1 & 2. Data were sourced from B.O.M. Strathbridge, Hagley Tasmania rainfall records (B.O.M. 2014)

2013								2014			
May	June	July	August	Sept	Oct	Nov	Dec	Jan	Feb	March	April
71	11	122	194	92	73	122	35	16	4	72	64

The rate of crop development was average for the district (Table 11). Site ECG10 exhibited greater than normal levels of onions in reproductive phase (bolting) at harvest, this lowered the yield figures as only commercially acceptable bulbs were sampled. Harvesting protocols on all sites were identical to Chapter 2. Samples were hand harvested from a 2-m^2 middle section of the centre bed of each plot and weighed to provide yield data for each plot.

Table 11. Site; planting date, population, lifting date, tops down % at lifting and growing days for the four trial locations.

Site	Planted	Population m^2	Lifted	Tops down%	Growing Days
ECG10	6/05/2013	79	23/12/2013	80	231
ECG20	8/05/2013	74	30/12/2013	90	236
RCG50	6/09/2013	70	6/02/2014	80	153
RCG60	12/09/2013	56	11/02/2014	90	152

Mature bulb assessment

Bulb assessment protocols were the same as Chapter 2 and were assessed at 30, 90 and 160-DAH. These periods correspond with the time of handling during commercial operation of initial handling (30-DAH), packing for export (90-DAH) and possible re-packing on arrival at export destination (160-DAH). In summary, at each assessment bulbs were subjected to handling stress using a tumbler adapted from Hole et al. (2002). A 200-litre polypropylene drum fitted with an access port and two internal rubber strips, was set at forty revolutions per minute for a period of ten minutes on Creamgold onions. Onions were able to move easily within the bag, and two bags were placed in the drum simultaneously. The tumbling method provided a consistent handling treatment across all plots and a relative estimate of a crops ability to withstand the rigours of packing and overseas shipping. Concurrent with this procedure ten randomly sampled bulbs, from each crop, were dispatched for testing to the Department of Primary Industry laboratory, Wagga, New South Wales, for soluble solids content (SSC) and Pyruvate levels for sensory analysis.

At 90 days and again at 160-DAH, samples were tumbled and assessed for skin loss for a second and third time. Bulb weight loss was measured at each assessment by weighing bulbs and weight loss was calculated by subtracting this value from the harvest weight. Weight loss associated with bulb disease was accounted for and these and affected bulbs were removed. Bulb disease is routinely assessed in grading and packing processes. Bulb breakdown is generally attributed to spp. Erwinia and spp. Pseudomonas, which is generally termed bacterial softrot and slipskin (Schwartz 2008) No *Botrytis allii* was observed at any crop stage.

Data analysis

Onion bulb yield and quality data were analysed in several stages by fitting linear mixed models using proc Mixed of SAS version 9.3. The sulphur by molybdenum factorial experiment and the ammonium sulphate trial were analysed separately but reported in the same tables and graphs, as they were integrated and shared the untreated control. Sulphur, Molybdenum and Site along with 2 and 3-way interactions were included as fixed factors and Block, and Block within Site were included as random factors. Block within Site was the random effect used to test Site effects and all other fixed terms were tested against the residual error. Nitrogen, Site and Nitrogen by Site were included as fixed factors with Block, and Block within Site included as random factors. The Block within Site random effect was used to test Site effects and all other fixed terms were tested against the residual error.

Results

Yield and cultivar

Bulb yields were not affected by the application of sulphur, molybdenum or ammonium sulphate, but did change by site. In this study, the four sites differed in soil type, crop management and microclimate. Yield ranged from 61.4 ± 2.6 t/ha^{-1} (mean ± 1 SEM) at ECG10 to 101.9 ± 2.6 t/ha^{-1} at ECG20 (Table 12). Onions at site ECG10 exhibited greater than normal levels of reproductive growth (bolting) by harvest. Consequently, this crop produced the lowest yield as only commercially acceptable bulbs were sampled at harvest.

Skin loss

30-DAH Bulb assessment

None of the treatments in these experiments affected skin loss ($p>0.05$) but there was a site affect at each post-harvest assessment date, 30, 90 and 160 days after harvest (DAH) (Table 12). At 30-DAH, an average of 10% of bulbs exhibited skin loss. The proportion of bulbs with skin loss at site ECG10 was 20% and significantly higher than that recorded at sites ECG20, RCG50 and RCG60.

90-DAH Bulb assessment

Skin loss increased more than two-fold at each site from 30 to 90-DAH (Table 12) and sites were ranked in a similar order on both assessment dates. The average level of skin loss across sites was 18% (Table 13).

160-DAH Bulb assessment

As with the earlier bulb assessments, site was the only influential factor (Table 12). The proportion of bulbs affected by skin loss were highest at site ECG10 and skin loss

at site RCG60 increased appreciably to reach a similar level. While the skin faults from ECG20 were the second highest at the 30-DAH assessment, this crop had the lowest level of loss at 160-DAH (Figure 6).

Treatment Summary

Table 12. Effect of site, sulphur and molybdenum and their interactions (Experiment 1) and the effect of site and nitrogen and their interaction (Experiment 2) on seven parameters: bulb yield, proportion of bulbs with skin defect at 30, 90 and 160-DAH, soluble solids content (SSC), Pyruvate concentration and dry matter percentage (DM). F is the F value and p is the probability. Num is the numerator degrees of freedom (DF) and Den is the denominator degrees of freedom.

Experiment 1

	DF		**Yield**		**30-DAH skin defect %**		**90-DAH skin defect %**		**160-DAH skin defect %**		**SSC %**		**Pyruvate mMol/ml**		**DM %**	
Factor	Num	Den	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Site	3	12	24.168	**<0.001**	23.901	**<0.001**	10.034	**<0.01**	53.378	**<0.001**	15.178	**<0.001**	46.809	**<0.001**	11.692	**<0.01**
S	1	36	0.004	0.947	0.522	0.475	0.074	0.787	0.021	0.884	0.001	1.000	16.920	**<0.001**	0.372	0.546
Mo	1	36	1.230	0.275	0.128	0.722	0.070	0.792	0.152	0.699	1.385	0.247	0.082	0.777	0.222	0.640
S*Mo	1	36	0.018	0.895	0.169	0.684	0.213	0.647	0.498	0.485	<0.01	0.942	0.736	0.397	0.444	0.509
S*Site	3	36	1.119	0.354	0.193	0.900	0.988	0.409	0.282	0.838	0.905	0.448	0.389	0.762	1.108	0.359
Mo*Site	3	36	0.910	0.446	0.051	0.985	0.290	0.832	0.244	0.865	2.500	0.075	2.606	0.067	1.135	0.348
S*Mo*Site	3	36	0.861	0.470	0.044	0.988	0.245	0.864	0.364	0.780	0.189	0.903	2.671	0.062	0.861	0.470

Experiment 2

	DF		**Yield**		**30-DAH skin defect %**		**90-DAH skin defect %**		**160-DAH skin defect %**		**SSC %**		**Pyruvate mMol/ml**		**DM %**	
Factor	Num	Den	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Site	3	12	14.647	**<0.001**	13.700	**<0.001**	10.746	**<0.01**	16.115	**<0.001**	16.115	**<0.001**	19.817	**<0.001**	4.445	**0.025**
AS	1	12	1.180	0.299	0.087	0.773	0.250	0.859	1.924	0.191	4.898	**0.047**	29.833	**<0.001**	0.183	0.676
AS*Site	3	12	1.501	0.264	0.754	0.541	0.250	0.859	0.141	0.933	0.986	0.432	2.630	0.098	0.247	0.862

Estimated Marginal Means

Table 13. Estimated marginal means at each site for yield (t/ha^{-1}), and skin loss (%) at 30, 90 and 160-DAH, soluble solids concentration (%), pyruvate (mmol/ml^{-1}) and dry matter (%). Letters within each column indicate sub grouping using Tukey's HSD (p=0.05). Pooled SEM is the pooled Standard Error of the Mean.

Experiment 1

Site	Yield	30-DAH skin defect (%)	90-DAH skin defect (%)	160-DAH skin defect (%)	SSC (%)	Pyruvate (mmol/ml^{-1})	DM (%)
ECG10	70.5^{a}	18.7^{b}	26.1^{b}	74.1^{c}	11.43^{bc}	4.94^{b}	13.8^{c}
ECG20	101.9^{c}	10.4^{a}	15.7^{a}	48.1^{a}	11.14^{b}	5.40^{bc}	13.4^{bc}
RCG50	99.9^{c}	7.6^{a}	16.5^{a}	64.0^{b}	10.75^{a}	3.66^{a}	12.8^{a}
RCG60	82.1^{b}	5.5^{a}	12.2^{a}	71.3^{bc}	11.59^{c}	5.74^{c}	13.0^{ab}
Pooled SEM	*2.63*	*1.31*	*1.87*	*2.41*	*0.08*	*0.14*	*0.14*

Experiment 2

Site	Yield	30-DAH skin defect (%)	90-DAH skin defect (%)	160-DAH skin defect (%)	SSC (%)	Pyruvate (mmol/ml^{-1})	DM (%)
ECG10	61.4^{a}	20.6^{b}	31.4^{b}	76.8^{b}	11.44^{b}	4.85^{a}	13.6^{b}
ECG20	100.7^{c}	9.0^{a}	13.1^{a}	49.8^{a}	10.97^{ab}	5.32^{ab}	13.5^{b}
RCG50	98.0^{c}	6.5^{a}	15.9^{a}	62.9^{ab}	10.61^{a}	4.67^{a}	12.7^{a}
RCG60	82.8^{b}	5.5^{a}	13.7^{a}	74.9^{b}	11.46^{b}	6.32^{b}	13.1^{ab}
Pooled SEM	*2.42*	*1.13*	*2.24*	*3.34*	*0.14*	*0.25*	*0.17*

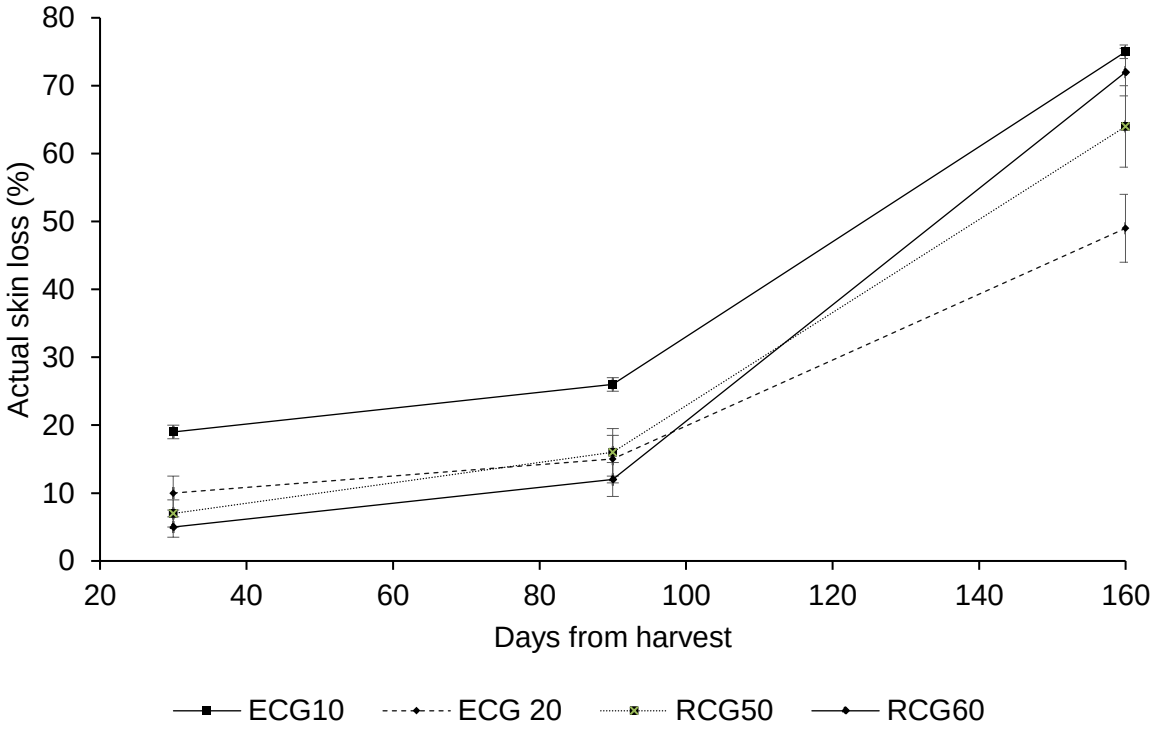


Figure 6. Mean percentage of bulbs with skin loss from handling assessment at 30, 90 and 160 days post-harvest, by site. Values are the mean from all plots at each site, error bars (± 1 SEM).

Sensory assessment

Bulb pungency

Bulb pyruvate concentrations differed among sites ($p<0.001$) ranging from 3.66 ± 0.14 mmol/ml^{-1} at site RCG50, to 5.74 ± 0.14 mmol/ml^{-1} at site RCG60. There was a main effect for both the sulphur trioxide and ammonium sulphate applications (Table 12). Sulphur trioxide application led to an increase in pyruvate concentration of 0.584 mmol/ml^{-1}. When ammonium sulphate was applied, the mean pyruvate concentration increased by 1.387 mmol/ml^{-1}, more than twice the response to sulphur trioxide (Figure 7).

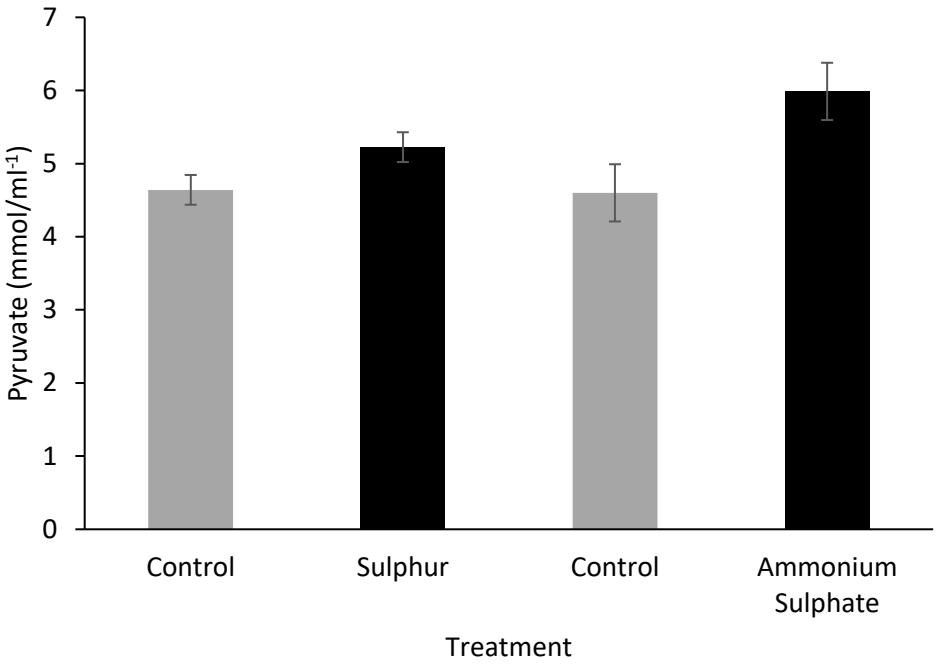


Figure 7. Mean Pyruvate (mmol/ml^{-1}) concentration in mature bulbs for untreated control and sulphur (Experiment 1) or ammonium sulphate (Experiment 2). Values are means of four replicates, error bars represent ± 1 SEM.

Bulb Soluble Solids Content

Onion bulb soluble solids content varied among sites (Table 12) however, neither sulphur nor molybdenum had a significant effect. Bulbs from plots treated with ammonium sulphate had lower soluble solids content ($p<0.05$) than the untreated control (Table 13).

Bulb dry matter

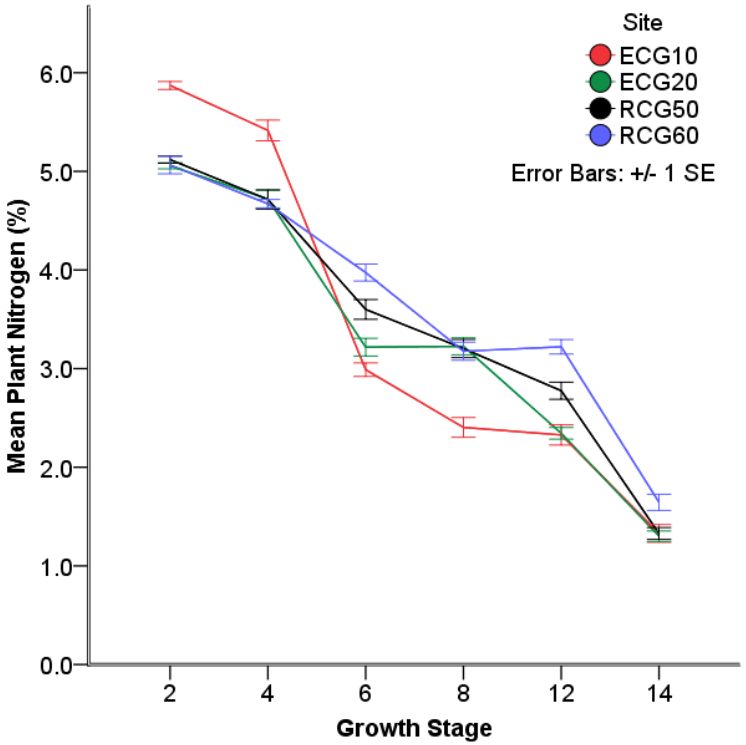
Bulb dry matter content did not respond to the application of sulphur, molybdenum or ammonium sulphate but was influenced by site in both Experiment 1 ($p<0.01$) and Experiment 2 ($p<0.05$) (Table 12).

Treatment effects on plant tissue concentrations

Nitrogen

The application of sulphur or molybdenum did not influence plant tissue nitrogen concentrations during crop growth or in the harvested bulb (Figure 8). Site had an influence on plant nitrogen concentrations which overall declined as crop growth progressed ($p<0.001$). Application of ammonium sulphate increased plant nitrogen concentrations across all growth stages ($p<0.01$).

a.



b.

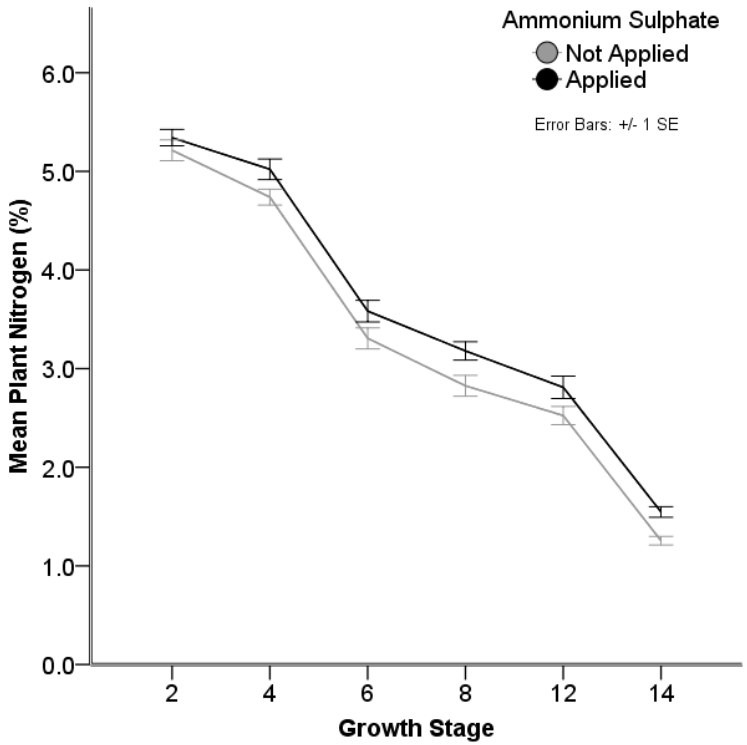
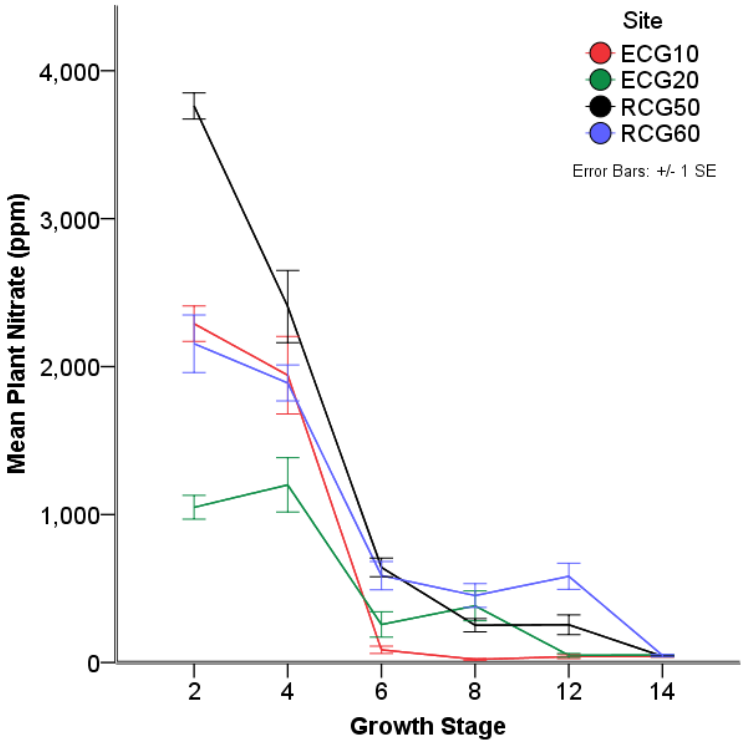


Figure 8. Experiment 2 Plant nitrogen concentration (%) from (a) 2 TL to mature bulb (14) by site and (b) from 2 TL to mature bulb (14) in response to ammonium sulphate amendment. Values are estimated marginal means, error bars represent ± 1 SEM.

Nitrate

Amendments of sulphur or molybdenum did not influence ($p>0.05$) plant nitrate concentrations (Figure 9) while the application of ammonium sulphate led to higher nitrate levels ($p<0.05$) during crop growth, but not in the harvested bulb (Figure 9). Site significantly influenced plant nitrate in both trials but did not affect bulb nitrate at harvest ($p<0.01$).

a.



b.

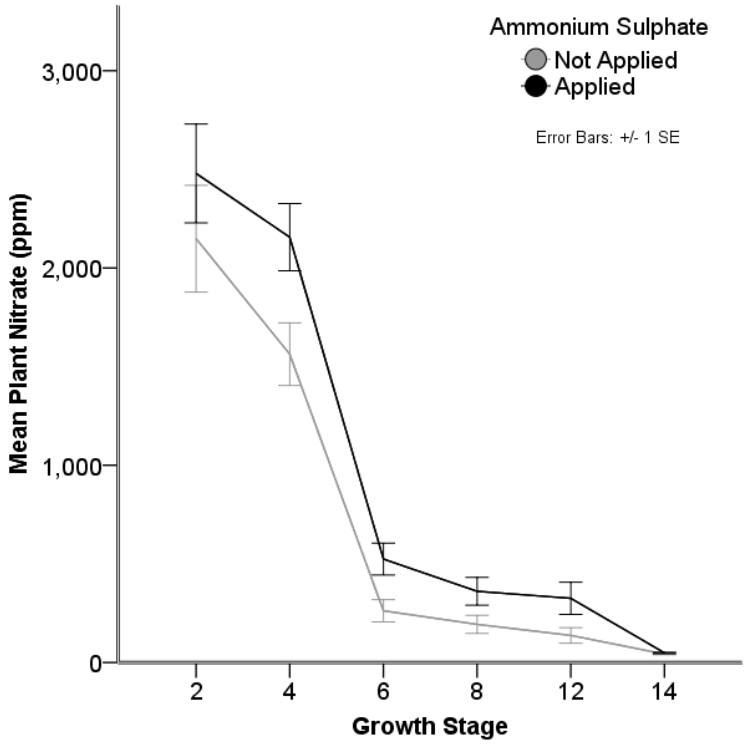


Figure 9. Experiment 2 Plant nitrate concentration (ppm) from (a) 2 TL to mature bulb (14) by site and (b) from 2 TL to mature bulb (14) in response to ammonium sulphate amendment. Values are estimated marginal means, error bars represent ± 1 SEM.

Sulphur

The application of sulphur trioxide or ammonium sulphate generally led to elevated levels of plant tissue sulphur concentrations; however, the extent of this was varied by an interaction with site (Figure 10). The effect of site was particularly evident at RCG60 on a Chromosol soil type. Application of ammonium sulphate produced a greater response than the application of sulphur trioxide. Tissue concentrations of sulphur also decreased as crop growth progressed. There was a clear response to treatment applications at 12 TL for the ECG sites and during the 4 to 6 TL at RCG site (Figure 10, Figure 11)

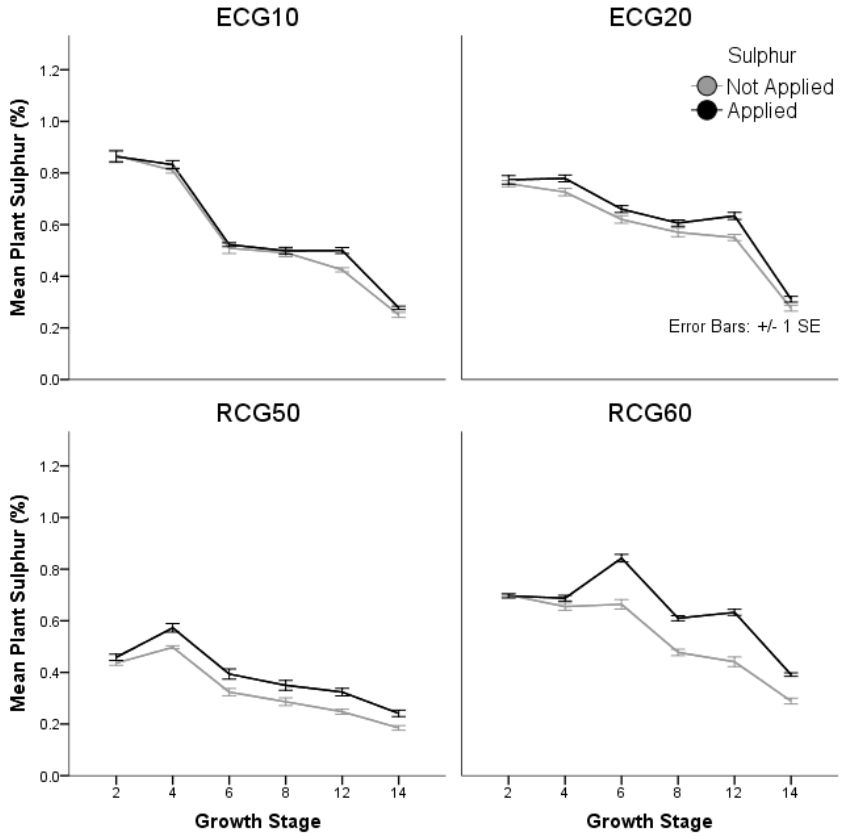


Figure 10. Plant sulphur concentration (%) from 2 TL to mature bulb (14) growth stage for each site, with responses to Experiment 1 (sulphur) amendments. Values are estimated marginal means, error bars represent ± 1 SEM.

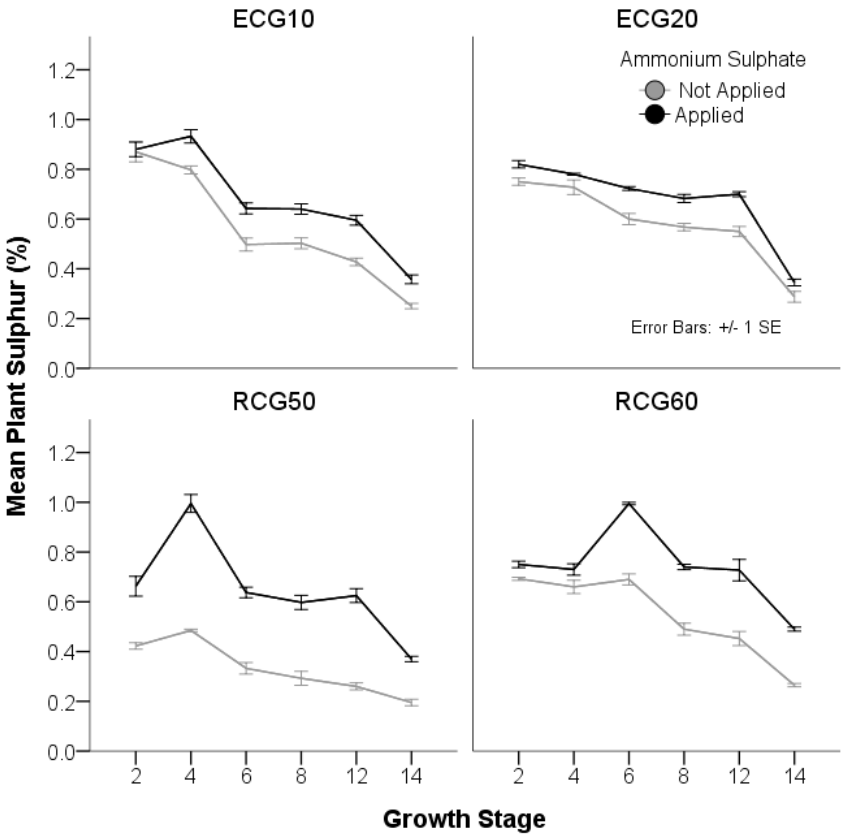


Figure 11. Plant sulphur concentration (%) from 2 TL to mature bulb (14) growth stage for each site, with responses to Experiment 2 (ammonium sulphate) amendments. Values are estimated marginal means, error bars represent ± 1 SEM.

Molybdenum

In Experiment one, there was a three-way interaction between molybdenum application, sulphur application and site ($p<0.001$). Molybdenum application led to elevated plant tissue Mo concentrations at site RCG60 and when sulphur was applied in addition to molybdenum the response was suppressed (Figure 12).

In Experiment 2, where ammonium sulphate was applied the concentration of plant tissue molybdenum was below the untreated control. The extent and timing of the response was moderated by an interaction with site ($p<0.001$) (Figure 13). Molybdenum tissue concentrations were extraordinarily high and varied widely at the 2 TL growth stages just after the application foliar molybdenum, suggesting application

residue on the surface of the leaves may have biased the laboratory results despite rainfall and irrigation. The results at this growth stage are therefore not reported. Against the control, molybdenum tissue concentrations were 30-fold higher at the 4 TL stage at site RCG60 and by bulbing, Mo tissue concentrations were 18-fold higher. The application of ammonium sulphate tended to reduce Mo tissue concentrations at varied growth stages (particularly at the RCG sites).

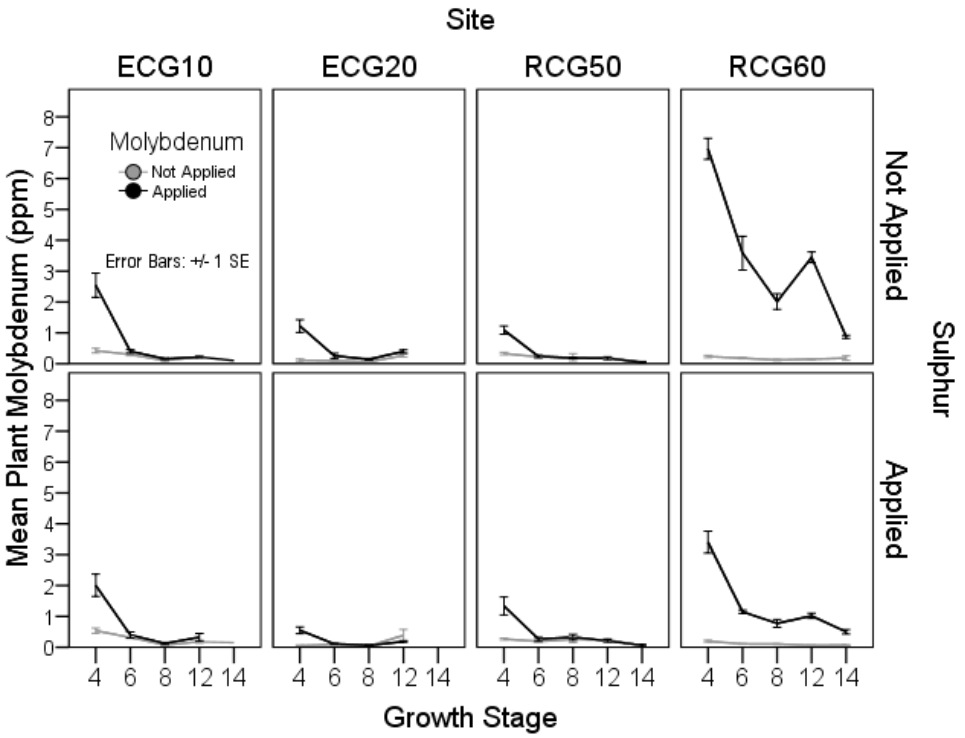


Figure 12. Plant molybdenum concentration (ppm) by growth stage and S/Mo factorial experiment from 4 TL to mature bulb (14) growth stage for each site with factorial responses to both molybdenum and sulphur amendments. Points not plotted reflect a not detectable response. Values are estimated marginal means, error bars represent ± 1 SEM.

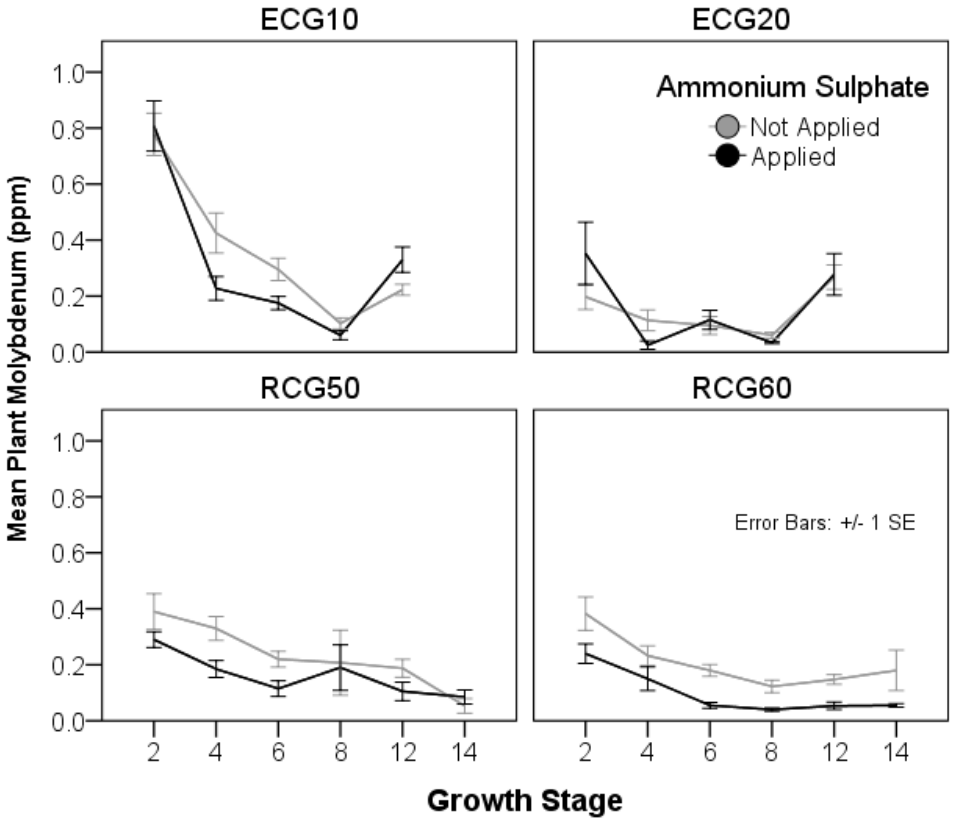


Figure 13. Plant molybdenum concentration (ppm) from 2 TL to mature bulb (14) growth stage for each site, with response to ammonium sulphate amendments. Points not plotted reflect a not detectable response. Values are estimated marginal means, error bars represent ± 1 SEM.

Discussion

A positive association between the nutrients nitrate, sulphur and molybdenum and the incidence of onion skin loss was recorded in the previous chapter from a survey of commercial crops. Using similar cultivars and soil types, this study sought to increase tissue concentrations of these elements through fertiliser amendments to evaluate a potential causal relationship. Although tissue concentrations of these elements were elevated above untreated control (commercial standard), there was no effect on onion bulb yield nor skin loss. Application of these elements did however influence parameters associated with sensory perception. Pyruvate concentrations were elevated in response to sulphur and ammonium sulphate application and, bulb soluble solid concentrations declined where ammonium sulphate was applied. Site, which represented an assimilation of cultivar, soil type and agronomic management, had the greatest influence in this study, altering responses in yield, skin loss and bulb sensory parameters.

Influence of molybdenum and sulphur application on skin loss

Establishing a causal relationship between crop nutrition and bulb quality in Tasmania would substantially enhance the successful production of bulbs suited to long-distance transport for counter season export markets. Earlier studies have linked nitrogen, phosphorus and potassium management with the post-harvest characteristics of mature bulbs (Kumar et al. 2007) and some evidence suggests that sulphur and molybdenum may also influence skin loss and therefore storage outcomes (Chapter 2). Bulb susceptibility to skin loss was measured over an extended period to match the timing and conditions of commercial export crops in ambient air conditions, by assessing skin loss at 30, 90 and 160 days after harvest. Despite the earlier evidence,

in-field applications of sulphur trioxide, molybdenum and ammonium sulphate did not influence bulb quality when compared with untreated control (commercial standard practice). The tumbling technique was developed to provide a consistent level of physical impacts in order to objectively assess onion bulb susceptibility to damage as expressed by skin splitting and cracking (Hole, Drew & Gray 2002). Overall using this technique, skin loss increased appreciably from 18% at 90-DAH to 65% at 160-DAH.

The loss of intact bulb skins during regrading and packing at Northern Hemisphere destinations has been highlighted as a major quality problem to Southern Hemisphere based producers and suppliers for many years. (Allwright 1993; Dennis et al. 2014; Gracie et al. 2006, 2012; Maier, Dahlenburg & Twigden 1990b; Wright & Grant 1997). This regrading and packing at Northern Hemisphere destinations usually occurs at approximately 160-DAH and the susceptibility of skin loss can vary substantially among crops as demonstrated in this study.

While the application of supplemental sulphur, molybdenum and ammonium sulphate did not influence bulb quality, bulb tissue concentrations of these elements were increased, in some cases substantially. Particularly striking was the 18-fold increase in molybdenum tissue concentrations for site RCG60 at the bulbing stage, and for which increased acquisition of this element was consistently higher than other sites for all growth stages. In the previous chapter, molybdenum concentrations in mature bulbs were linked by recursive partitioning analysis, with skin loss increasing where bulb dry matter was <12.4% and molybdenum concentrations >0.047 ppm. In this experiment, skin loss were not linked to molybdenum levels, which were higher than this threshold. This contrasting result is possibly because this threshold was the second step below dry matter, which did not fall below 12.4% as in the second chapter.

Plants are known to differ in their ability to extract molybdenum from the soil, with onions generally only absorbing low concentrations (Purvis 1955). Data from the previous chapter were consistent with this finding with only low concentrations of molybdenum detected in both leaf and bulb tissue (0.08 ppm), fifty-fold lower than the 4ppm observed at bulbing growth stage from site RCG60 (Figure 12).

This response was difficult to reconcile with the current understanding of molybdenum acquisition. Availability of molybdenum anions in the soil solution are mediated by the presence of positively charged aluminium and iron oxides, soil pH, organic matter and soil moisture (Barker 2016; Kaiser et al. 2005; Vistoso et al. 2009). Molybdenum availability is increased by $pH_{(CaCl)}$ above 5.5 and higher organic matter decreases the amount of the ion bound by metal oxides (Rutkowska et al. 2014). Crop RCG60 was planted on a Chromosol soil type, which had the lowest pH, organic matter and cation exchange capacity (CEC) of the four sites. From this, we would have expected that the availability of molybdenum in the soil solution in this soil type to be the lowest. This contrasting result may be due to the limited understanding of how plants adsorb molybdate from the soil solution and then distribute it once in the plant (Kaiser et al. 2005).

Increasing concentrations of nitrate and sulphur were linked to skin quality in the previous chapter, where increased skin loss were associated with nitrate >20ppm as the primary step, and sulphur >0.34% as a second step (recursive partitioning) in the mature bulb tissue. In Experiment 1 of this study, treatment applications of S at 90kg/ha^{-1} were applied in addition to a pre-plant amendment of S at 90kg/ha^{-1} to the ECG crops, and 4.5kg/ha^{-1} of S pre-plant application to the RCG crops. Neither of these treatments influenced bulb skinning, possibly because bulb sulphur and nitrate tissue concentrations were lower than these postulated skin loss thresholds. In

contrast, application of ammonium sulphate (S at 108kg/ha^{-1}) in Experiment 2 did raise the sulphur tissue concentration of mature bulbs over the 0.34% threshold at RCG60 but this did not result in any detectable increase in susceptibility to skin loss. Chapter two data demonstrated that RCG crops were less susceptible to skinning than ECG crops suggesting further study is required to understand the effect increasing concentrations of sulphur and genotype has on the quality and skin loss of onions.

Influence of sulphur and molybdenum application on sensory perception

Sulphur's role in onions has largely been studied as a component of an onion's flavour profile (Crowther et al. 2005) for example its effect on pungency, total soluble solids or dry matter (McCallum et al. 2005; Randle, Kopsell & Kopsell 2002; Randle et al. 1999; Randle et al. 1995). In this study, a significant relationship was established between pyruvate levels in mature bulbs and increased applications of sulphur trioxide and ammonium sulphate. It has been documented that onion flavour can be modified by sulphur fertility where more available sulphur results in higher pyruvate concentrations (Bolandnazar, Mollavali & Tabatabaei 2012; Lancaster et al. 1988). The association between sulphur nutrition and onion pungency has largely been derived from glasshouse studies, with a limited number of field-based studies being able to duplicate this response (McCallum et al. 2005). While this relationship is accepted, differences between total bulb sulphur content and pungency among onion cultivars ranging from sweet to pungent suggests that both genotype and environment play the primary role in pyruvate concentration (Ketter & Randle 1998). Interestingly, the application of sulphur increased the bulb pyruvate concentrations in both the ECG crops where high amounts of sulphur were applied pre-plant (S at 90 kg/ha^{-1}) and in the RCG crops where much lower amounts of sulphur (4.5 kg/ha^{-1}) were applied pre-plant. This response was greater in the RCG than the ECG sites (Table 13) possibly

due to moderation from the pre-plant sulphur application. Compared to sulphur trioxide, the application of ammonium sulphate appeared to be more efficacious, increasing bulb pyruvate concentration by twice as much. This suggests that where producers would like to increase bulb pungency, the use of ammonium sulphate may be more effective.

Influence of genetics, site and seasonal conditions

The genetics of an onion cultivar are reported to have the most influence on bulb skin characteristics (Hole, Drew & Gray 2002). Some onion cultivars consistently produce bulbs with five to six skins whilst others only produce an average of two skins (Allwright 1993; Gracie et al. 2006). This was supported by a UK study where cultivar selection substantially influenced skin quality and number, independent of environmental and agronomic factors (Hole, Drew & Gray 2002). Bulb skin quality derived from genetic attributes can be further enhanced by careful post-harvest treatment, combining both controlled heat and the correct level of humidity (Chope et al. 2012). Despite these studies determining the importance of cultivar and curing, the physiological basis of the skin formation process was not clearly understood. Galsurker *et al.* (2016) provided new evidence that desiccation of the scales in conjunction with senescence, resulted in programmed cell death and formation of the characteristic brown colouring of the outer scales. The latest research suggests this skin forming process was not observed on inner bulb scales, despite lengthy heat treatment. This work suggests that a differential response was possible between outer and inner bulb scales from heat stress, and the inner scales can show resistance to skin formation (Galsurker et al. 2018).

Given the closely related seed lines used in this study, the most influential factor was the commercial site which substantially influenced all key response variables (Table 12). Other authors have also noted the strong influence of site on the growth and development of onions, and the stark contrast that can occur paddock to paddock even though growing conditions and soils are similar (Costigan, Greenwood & McBurney 1983). Onion crops of the same genotype regularly vary in skin quality, this also indicating that agronomic practices and environmental conditions play a key role (Ariyama et al. 2006; Costigan, Greenwood & McBurney 1983).

Differences in crop outcomes are also influenced by season with bulb quality and skin number changing from year to year (Hole, Drew & Gray 2002). Nutrient acquisition by onion crops has been observed to change within a season (Westerveld et al. 2003). In this study, there was a peak in sulphur tissue concentration at the 4-6 leaf stage in the RCG sites, and mid-bulbing at the ECG sites. Although these peaks occurred at different growth stages, all occurred between the 10th to the 20th December, indicating this may have been a seasonal response, possibly due to temperature. This influence of uncontrollable variables highlights the complexity of establishing relationships between plant nutrition and soil amendments to crop outcomes. The influence of site in this study is an effective illustration that the combination of the uncontrolled variables may often play a role that overrides treatments. This is common to in-field experiments and many other investigators have noted unexplained variances attributed to soil or climatic factors overriding treatment effects (Barnes, Greenwood & Cleaver 2009; Ferguson & Watkins 1992; Greenwood et al. 2009).

This study has shown that improving onion skin quality is not necessarily entrained to the manipulation of soil nutrition and that uncontrolled variables associated with site can have a far greater influence. Our attempt to alter bulb nitrogen metabolism using

supplemental applications of sulphur, molybdenum and ammonium sulphate did not affect bulb skin loss, the major determinant of export bulb quality. This is contrary to data from our earlier survey study, which suggested that the excess application of sulphur would influence nitrogen metabolism and increase skin loss. The tumbling process at 30, 90 and 160 days after harvest was able to differentiate between crop susceptibility to skin loss indicating that this process is a potentially useful commercial tool to rank bulb robustness.

General Discussion

Introduction

The motivation for this study was to improve our understanding of Tasmanian Creamgold onion crop nutrition. Successfully adding to existing knowledge will enhance sustainable export bulb production as the paucity of information offered for nutrient management of this important export crop presents an increased production risk.

Most commercial onion nutrition programmes are subjective and based on individual experiential knowledge. A review of the published literature on levels of plant tissue concentration for optimal onion production indicated that threshold recommendations have been sourced mainly from research studies conducted in the Northern Hemisphere. These studies used different cultivars than those commonly cultivated in Tasmania (Zink 1962, 1966). Some data have been referenced from Australian research conducted from the 1960's to 1980's, but the trial latitudes suggest the cultivars may have had different daylength requirements compared to intermediate daylength production in Tasmania (Reuter & Robinson 1997). More recent Australian onion nutrition studies have been conducted, albeit these had a specific focus on a limited range of macronutrients (Allwright 1993; Maier, Dahlenburg & Twigden 1990a, 1990b, 1992).

To successfully monitor onion crop nutrition requirements through key growth and development stages requires regular assessment of plant nutrient levels (Brewster 1989). The use of plant tissue analysis for this purpose has been limited, however, due to a paucity of suitable reference values for the critical nutrient concentration required for optimal growth (Ekbladh 2007). This study has expanded on previous

research by considering macro and micronutrient plant tissue levels from seedling to harvested bulb, and linking these to potential effects on bulb yield and quality in a commercial setting. Establishing the nutrient concentrations at all key growth stages and linking this information to bulb quality has the potential to optimise the production of intermediate daylength Creamgold crops in the Southern Hemisphere. This study adds to the scientific understanding of the range of nutritional element concentrations found in high yielding onion crops, complementing existing data, and contributing new data for elemental tissue concentrations at key growth stages not previously reported.

Research implications

This study highlights the importance of considering the holistic effects of crop nutrition on yield and quality in a commercial context. Many nutritional studies have placed an emphasis on yield. In contrast, this study also considered the effects of crop nutrition on bulb quality following long-term storage.

The large-scale survey of 34 commercial crops enabled benchmarking of the current nutritional status of Tasmanian Creamgold onion production. To the knowledge of the author this approach is unprecedented in onions and the survey of the commercial crops from 2 TL to harvest generated significant data for commercial management use while also enabling exploration of potential links between nutrition and key bulb quality attributes. The survey findings showed a lack of correlation between soil and plant tissue concentrations for the majority of nutrients, with the exception of some Group 3 elements. This was possibly due to the dynamic and complex interactions between soil and plants apropos to nutrient availability, acquisition and partitioning (Boyhan, Torrance & Hill 2007; de Visser, Van Den Berg & Niers 1995). This is consistent with the findings of Marschner (2011) who reported the amount of nutrient adsorbed by a

plant from the soil depends on nutrient group, soil type, plant and environmental factors.

The lack of relationship between plant or soil nutrient levels and bulb yield suggests that nutrient concentration has not limited growth and development, and this is consistent with field observations where with the exception of nitrogen during bulbing, no visible symptoms of nutrient deficiency or excess were noted. This occurred even though some nutrients would be classified as deficient according to current recommendations (Reuter & Robinson 1997). Nitrogen deficiency symptoms are expected to occur during bulbing as nitrogen amendments are purposely withheld to induce this deficiency to maximise storage life (Wright 1993). Plant tissue concentrations of the Group 3 nutrients, calcium, magnesium and chlorine, were lower than previously published thresholds for sufficiency. On this basis, these lower levels are most likely representative of a lower requirement for these nutrients than previously thought.

We have reported the 5th and 95th percentile of each nutrient across all key stages of growth and development. This data includes early and late plantings and the key soil types present in the production district. Knowledge of this range provides a framework that can be used by agronomists and growers as a guide for understanding the relevance of tissue nutrient levels at key stages of development, and with further refinement could be used to enhance onion crop fertiliser programs for different soil types and production systems.

Site had the greatest influence on all the measured attributes and highlighted that manipulating crop nutrients in a field setting is challenging, with the crop outcomes often primarily determined by soil type, agronomy and seasonal influences (Agnieszka

et al. 2017; Allwright 1993). The influence of site has been reported by other authors and the stark contrast that can occur between paddocks even with similar soil types (Costigan, Greenwood & McBurney 1983). Many attempts have been made to unravel the influence site factors have on experimental treatments albeit our capacity to achieve this is perhaps limited by the number of strong factors present within any field site. These factors increase the complexity of the interactions between treatments and/or inputs and the crop plants. Independent variables often present in field situations include air and soil temperature, solar insolation, and the intricacies associated with both soil and plant dynamics (e.g. soil biology). The import of season to season variance in crops has been well recognised (Allwright 1993; Lancaster et al. 1995; Lancaster et al. 1996). Comparing this work to other significant longer-term studies that have resulted in a regional change of practice, suggests additional onion nutrition research would be beneficial to capture the seasonal effect (Boyhan & Kelley 2007; Boyhan, Torrance & Hill 2007; Boyhan et al. 2014).

The loss of bulb skins to expose the underlying scale tissue is the main quality challenge for exported onions and has been at the forefront of onion research conducted previously in Tasmania (Allwright 1993; Gracie et al. 2006). Work in both Northern and Southern Hemispheres have demonstrated seasonal and agronomic effects on the expression of skin loss susceptibility (Gracie et al. 2006; Hole, Drew & Gray 2002). In both these studies the susceptibility to skin loss was assessed using a tumbling technique to provide a consistent level of impact to all bulb samples that mimics commercial handling of bulbs. This study utilised the same technique but also assessed bulbs at key times consistent with existing commercial handling: initial grading (30 DAH), packing for shipping (90 DAH) and final repacking and sorting (160 DAH) at Northern Hemisphere export destinations (Gracie et al. 2006). The tumbling

impact on bulbs at each assessment demonstrated increasing susceptibility to skin loss as storage time progressed. This finding suggests that bulbs should be packed and shipped before 90 DAH. The apparent rate of increase between this assessment and 160 DAH suggests that handling should be kept to a minimum after shipping.

Analysis of the survey data revealed that increased levels of skin loss were associated with elevated tissue concentrations of sulphur, molybdenum and nitrate in mature bulbs (Chapter 2). There is a paucity of reference data relating to concentrations of these elements in onion tissue during most stages of growth and development in the Southern Hemisphere (Reuter & Robinson 1997).

Of these, sub-optimal or super-optimal concentrations of nitrate have been linked to many physiological disorders in high value horticultural crops. Examples of this include hollow stem in broccoli (Belec et al. 2001) poor fruit colour in apple and cranberry, small fruit size in orange (Kays 1999) and low specific gravity of processing potato tubers (Zebarth et al. 2004). The link between nitrate and these physiological disorders may be explained by a plant growth and development response attributed to a signal from nitrate. Reported by one author as a growth regulator effect (Trewavas 1983) this signal can alter the plants metabolism (Crawford & Glass 1998). The assimilation of nitrogen in plants via the conversion of nitrate to ammonium is mediated by the bio-chemical pathways catalysed by nitrate reductase (Crawford & Glass 1998; Masclaux-Daubresse et al. 2010). Other important components of nitrate transport within the plant are nitrate loading into the xylem and nitrate uptake in the shoots and leaves (Tischner 2000). All the elements identified in this study that contributed to bulb skin loss, are also entwined in the nitrate reductase process.

Molybdenum has a key role in the nitrate reductase dimer complex (Taiz & Zeiger 2010). The plant tissue survey results from this study indicated that molybdenum concentrations in Tasmanian onions were generally at low to not detectable (<0.01ppm) levels, with increased concentrations contributing to skin defects. Molybdenum is also involved in plant sulphur metabolism (Hänsch & Mendel 2009) through a reaction altering the function of the protein (Schwarz & Mendel 2006). Similarly, a sulphur-iron cluster is a prosthetic group of the enzyme nitrite reductase, this enzyme facilitating the conversion of nitrite to ammonium (Taiz & Zeiger 2010). Molybdenum uptake into root cells can either be enhanced or alternatively supressed by concentrations of sulphur in the growing medium (Alhendawi, Kirkby & Pilbeam 2005). This is known to occur as sulphate and molybdate anions both enter through the same proton-coupled symporters (Kaiser et al. 2005). Although the research by Fitzpatrick *et al.* (2008) concluded that suppressed molybdenum uptake may also be a response to regulated expression and activity of the sulphate transport and assimilatory pathways despite increased sulphur concentrations in the growing medium.

We postulated, albeit reductively, that as nitrate levels increased in the plant tissue the quantities of both molybdenum and sulphur required for its metabolism may have also increased in concert. Based on the survey data, we hypothesise that elevating the concentration of sulphur and molybdenum in plant tissue would lead to increased skin defects. As both molybdenum and sulphur are also required for nitrate metabolism within plants, thus their effects on skin defects may be indirect, which would support the experiment outcomes. Increased nitrate concentrations would have facilitated growth rate, possibly through increased cell volume and a subsequent increase in bulb moisture content. Hence, the increase in skin loss associated with these elements and

bulb moisture may reflect increased growth rates resulting in increased circumferential tension and a resultant failure in skin tissues.

Application of additional sulphur, molybdenum and ammonium sulphate to crops during early growth and development increased tissue concentrations of the constituent elements. This positive association between each of these nutrient amendments and susceptibility to bulb skin loss was assessed across four different soil types using controlled experiments. Despite the elevated tissue concentrations, the treatments did not have a significant effect on skin loss.

The presence of downy mildew during onion growth and development has been shown to affect skin mechanical properties (Gracie et al. 2006, 2012) and it was postulated that downy mildew infection damages tissues of the leaf sheaths that later form skins. This earlier study identified visible signs of spores on foliage contributing to skin loss, which based on the classification from this study would have an observational rating of 6 (severe mildew – multiple leaves covered in spores) (Gracie et al. 2006). Here an observation rating of 3 (no disease evident / conditions suitable for infection) and increased nitrate were linked to susceptibility of bulbs to skin loss. This suggests the leaf sheath tissues of the developing onion plant may be vulnerable to environmental influences in addition to attack from fungal infection. The ability to assess crop susceptibility to skin loss and potentially relate this to actual shipping losses would advance our understanding of this industry problem.

Pyruvate and bulb soluble solids, important sensory properties, were affected by the application of sulphur compounds. Two sulphur compounds, sulphur trioxide and ammonium sulphate, were applied to plots and both led to higher levels of Pyruvate with the application of ammonium sulphate increasing the concentration by twice the

net change. This suggests that where producers are cautious about bulb pungency for consumers, the use of ammonium sulphate may need to be minimised. Ammonium sulphate applications have been reported to lower bulb soluble solid content and the reduction may increase bulb moisture (Lin, Watson & Baggett 1995). Therefore, applications of ammonium sulphate may increase pyruvate concentration, lower SSC, change the sensory perception and quality of a Creamgold onion cultivar.

Recommendations

This study has advanced our understanding of onion crop nutrition in Tasmania. The survey of commercial crops across all key stages of growth and development has provided a benchmark that the industry can build on. The key findings from the survey were further investigated and the outcomes demonstrate the need to consider long-term storage, and the indirect impacts on quality parameters such as bulb moisture, pungency and soluble solid content.

To advance this body of work I recommend:

Sites used in this study varied in soil type, time of sowing and agronomy practices. It was impossible to separate their individual effects on the onion characteristics recorded and these parameters can combine to have substantial effect on crop quality of similar cultivars (Boyhan et al. 2014; Costigan, Greenwood & McBurney 1983). To address this paucity in understanding we propose a study that assesses the individual components. An initial study that considers the effects of temperature and temperate gradients (between soil and air) have on growth and development processes of onion crops would provide basic information to permit improved interpretation of agronomic and site-specific studies and how these parameters interact.

Previous studies of other crop species have observed that both root and leaf temperatures change the dynamics of nitrogen acquisition (Frota & Tucker 1972; Moraghan & Porter 1975). A controlled study that evaluated diurnal temperature fluctuations with nitrogen application and uptake showed how the two parameters interacted to affect crop growth and development (Ewart & Kliewer 1977). Modelling of crop weight gain based on nutrient uptake and plant growing conditions of weather,

soil type, cultural practice and evapotranspiration was conducted in the UK (Greenwood & Karpinets 1997). A similar approach for onions would aid in improving our understanding the important site factors. We recommend that this would best achieved using a series of controlled environment experiments that manipulates shoot and root temperature. The approach would improve our understanding of the dynamic nature of nutrient acquisition and provide important information to underpin recommendations for adjusting fertiliser application based on seasonal conditions.

The intensive nature and scope of this study did not permit refinement of the crop survey parameters. A further study would incorporate soil profile assessment to establish the root depth of onions within the structure and therefore nutrient availability within that depth. This would enable better understanding of the management of inputs by soil and production type that may potentially have a positive effect on bulb quality.

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